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A TREATISE ON TROPICAL THERAPEUTICS

VOLUME ONE

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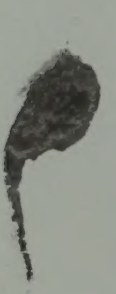
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PREFACE

The favourable reception accorded to the first edition of this book, which was published in 1936, though gratifying to the author, was offset by the fact that the book remained out of print for several years. In spite of the pressing demand by the post-graduate students and by the practising physicians, it has not been possible to bring out a new edition earlier. The constant demand for urgent work, the confusion caused by the World War II and the difficulty in getting paper were some of the factors responsible for this delay.

During the intervening decade, considerable progress has been made through systematic research in tropical medicine and therapeutics and quite a large number of drugs of great value has been introduced for the treatment of different tropical diseases. In fact, so rapid has been the progress that physicians find it increasingly difficult to keep pace with it. Moreover, the importance of appreciating the pharmacological action of drugs as the basis for their scientific use is becoming more apparent in the practice of modern medicine than in any other time before. Recognising, therefore, the immensity and importance of the rapid growth of Therapeutics and with the objective of making available all the useful information in one book, which can be used both as an advance text book and as a work of reference, the present edition has been largely re-written. In fact, almost every page has received attention and several new chapters and sections have been added, so that this edition may be regarded as an entirely new work. In addition to the therapeutic advances, clinical aspects of various disease conditions, in which drugs are recommended to be used, have been incorporated in this edition in a little more detail with a view to bring home to the physicians the exact methodology of their administration in clinical work. While every effort has been made to keep the text abreast of the modern advances in the subjects discussed, it should be realised that the progress has been so rapid, especially with regard to several chemotherapeutic

agents and antibiotic drugs, that a book becomes almost out of date by the time it leaves the press. It has, therefore, been decided to include further newer developments at the end of the book as an Addendum.

It is a pleasure to acknowledge the help that the senior author has received from Drs. B. Mukerji and I. C. Chopra, both his former students and co-workers at different periods. The contribution and helpful co-operation received from them have been of great value. In fact, it would not have been possible to bring out this edition without their active help and this is best acknowledged by including their names as joint authors. Thanks are also due to Drs. S. Bhattacharya, S. P. De, J. R. Kohli and many others, for active assistance in checking the proofs, etc.

The latest findings of science are always based on previous work of innumerable investigators. Therefore, it is obviously difficult and sometimes impossible to give full credit in all instances to every one concerned. The authors have freely consulted the writings of many of these contributors. Our acknowledgements are herewith extended to all of them and full credit has been given to them, whenever possible, in the text.

To facilitate early publication of some of the important matters and to meet the growing demand, the book is brought out, initially, in two volumes. It is intended eventually to combine them into one volume with full Index and references, when the second volume is ready.

Criticisms and suggestions for improvement will be gratefully received.

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June, 1950

PREFACE TO THE FIRST EDITION

Since 1921 I have been engaged in teaching post-graduate students at the Calcutta School of Tropical Medicine and nearly a hundred medical practitioners from all parts of India and some from other tropical countries have annually passed through my hands. Medicine is taught in the Medical Schools and Colleges of India, chiefly from British and American text-books where the climatic and the morbid conditions are somewhat different from those in the tropics. It is not surprising, therefore, that the therapeutic methods described therein are not universally applicable in the environment under which the medical practitioners work in the tropics. It is not unusual to meet with disappointment in results and even untoward effect from the applications of such methods.

The importance of this fact has impressed me so greatly that I have thought it worth while to review the whole subject of therapeutics with due regard to the climatic conditions met with in the tropics generally and in India particularly. All the experience gained during the last fifteen years of intimate association with post-graduate teaching and treatment of patients in a well-equipped research hospital such as the Carmichael Hospital for Tropical Diseases has been collected and put together in book form so that the information is readily available. To encourage the rational use of drugs, the pharmacological actions of various remedies have been described before their therapeutic uses are discussed. The ætiology and pathology of disease have been mentioned briefly only in so far as it is necessary to make the treatment more comprehensible. These additions have made the book somewhat bulky.

I would like to emphasize that not only does this book include the results of my own personal experience, but that of many of my colleagues and co-workers in the Calcutta School of Tropical Medicine, who have very generously helped me in producing this volume. Had

it not been for this co-operation and help the task would have been an impossible one. Every section of the book has thus been thoroughly scrutinised by various experts on particular subjects, who have liberally contributed valuable criticisms and suggestions, which have been incorporated. In this connection I would like to put on record my very great indebtedness, my sense of gratitude and appreciation for the help which has been ungrudgingly given me by everyone concerned.

* * * * *

The book is so designed that it will not only serve as a reference book for medical practitioners in the tropics but will also meet the requirements of senior students appearing in their final examinations. In spite of the great care that has been taken, the book possibly has many shortcomings and blemishes. I shall be very grateful for criticisms and suggestions so that in the next edition these defects can be removed.

R. N. CHOPRA.

Calcutta.
School of Tropical Medicine,
April, 1936

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PART I

GENERAL CONSIDERATIONS IN THERAPY

CHAPTER I

ACTION OF DRUGS

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1. Source and Nature of Drugs

Historical.

The problem of cure of disease has faced mankind as far back as memory can take us and he has looked for remedies to alleviate his sufferings from the earliest times. The evolution of drugs is traceable to the desire of man to overcome personal discomforts arising from disease. In course of time the knowledge of drugs and with it their number and uses have gradually increased. Although modern science has opened up new sources of remedies from minerals and the animal kingdom, reliance is still placed upon plant life for the supply of many drugs. The plants may occur in the wild state or they may be cultivated; they are collected and active preparations are made from them. The very name drug comes from the Anglo Saxon word *drugon* meaning to dry. Crude drugs are the commercial form of plant drugs as they are brought to the market. They yield one or more definite chemical bodies of medicinal value which are known as active principles or active constituents. These constituents may occur in the whole plant or in one particular part, *e.g.*, leaves, roots, seeds, etc.

Synthetic drugs.

Besides drugs of vegetable origin, there is a large group of drugs known as chemicals. Many chemical compounds are produced by plants, by natural processes at ordinary temperature and pressure, *e.g.*, alkaloids, glucosides, salts, etc. To produce similar compounds artificially, the chemist has to resort to powerful reagents such as strong acids and alkalis, large variations in temperature and pressure, distillation, fusion and many other drastic operations. Many of the products of the chemist are similar in action and composition to natural compounds, but they are cheaper to produce. Other chemicals belonging to the inorganic class are produced from natural minerals, sometimes by simple processes at other times by complicated ones. Then there are the comparatively recent remedial agents obtained by various chemical operations, mainly from

coal tar as well as other products obtained by the more or less complicated processes of synthesis. There are also the so-called organometallic compounds which form a powerful and important group, particularly in the treatment of protozoal diseases. These compounds have to be prepared with extreme care, as many of them are positively dangerous, unless they are in a state of absolute purity.

Biological products.

Biological products, such as vaccines, sera, gland products, hormones, bacteriophages, penicillin and other anti-biotics, etc., form another group of remedial agents. They require even greater skill and more expert knowledge than any other type of preparation used in medicine.

2. Action of Drugs as Basis of Therapy

Pharmacology.

Pharmacology is the term applied to the science which deals with the action of drugs upon animal organisms without special indication to their application in disease. It, in fact, denotes the physiological action of drugs, which forms the basis for their scientific use in curing diseased conditions, the alleviation and prevention of pain and prolongation of life. Experimental pharmacology deals with the reactions of living material to changes in environment, these changes being produced by drugs.

Therapeutics.

Therapeutics has been defined as the art and practice of treating abnormal states by any method that relieves pain, restores health or prolongs life. It comes from the Greek word *therapeutikas* meaning healing, curative, alleviative. It includes all remedial agents and measures used in the treatment of disease.

In old days treatment was largely symptomatic and the name allopathy was given to it. Remedies such as purgatives, blood-letting, etc., were used to suppress the symptoms of diseases. This was followed by the study of drugs upon normal individuals and the experimental science of pharmacology was born.

Therapeutics may be empirical or rational. The former includes any measure which experience has shown to be beneficial but whose action cannot be explained, rational therapy on the other hand consists in using drugs whose mode of action is understood.

Rational therapeutics.

The foundation of rational therapeutics was laid in the second half of the nineteenth century with the development of the science of pharmacology and critical study of the action of drugs on the functions of the body. It was considerably helped by rapid advances made in organic chemistry and production of powerful chemotherapeutic agents. Biochemistry also developed side by side, and insight into the role of vitamins, endocrines, etc. led to their use in the treatment of disease. During the last three decades the science of therapeutics has been developing on sound lines, and while great advances have already been made, more are expected in bringing diseases under control.

Healing force of nature.

In the treatment of disease full use should be made of all the measures available. In any improvement produced in the condition of the patient by the application of these measures the part played by nature must not be forgotten. Nature is infinitely wiser in the art of healing than we imagine. Variations from normal in disease are often an effort on the part of nature to meet the needs of the patient and should not always be considered as harmful. Proper use of therapeutic measures renders important help to these forces in curing disease in the least time and with least harm to the organism. The task of the physician consists in directing the treatment in such a manner as to remove the obstacles

which hinder the path of natural cure. This should be done by a thorough comprehension of the diseased conditions, that is by diagnosing the case as carefully and as fully as possible. Before ordering a drug or method of treatment, the physician should have a clear conception of what he is trying to accomplish. Drugs should not be prescribed unless there is a distinct indication for them. The doses of all the remedies should be very carefully considered, to fit the needs of the patient's condition. Special regard should be paid to such influential factors which have an important bearing on the treatment of disease as, the maintenance of the vitality of the patient by proper feeding, elimination of toxins by the kidneys, bowels and skin, relief of annoying symptoms, and giving to the patient sufficient physical and mental rest and sleep.

Guiding principles.

Physicians should not rest content with the relief of symptoms alone but should strike at the root cause of the disease. Unfortunately, for many of the diseases attacking man, specific remedies do not exist. The progress of chemotherapy during recent years has however, produced a large number of effective remedies against helminthic, protozoal and bacterial diseases. In this the commercial manufacturing organizations have played a very important part.

Commercial repercussions.

Unfortunately effective new compounds are at first patented by one concern but in course of time rival manufacturers evade these patent rights by slightly altering the formulae and giving their modifications new names. The natural result is a confusion in the mind of the practitioner who is presented with a host of proprietary labels covering compounds which possess almost the same therapeutic action. Extensive advertisement, which is a normal feature of modern drug concerns, is sometimes lent to remedies which have no proved value. This practice makes it incumbent upon the part of medical men to use only those remedies, the composition and action of which they have knowledge.

3. The Mode of Action of Drugs

Effective concentration.

It has been amply proved by modern research that many drugs act in very high dilutions. The unit dose of such compounds is expressed in fractions of a gamma (1 mgm=1,000 γg), and some substances produce effects on isolated organs in dilutions of 1 part in 10,000 million. As there are 6×10 molecules in a gram molecule of any chemical, a solution of adrenalin at a dilution of 1 in 10,000 million contains about 10,000 million molecules in a drop. It is therefore not correct to assume that science of chemistry and its laws have no bearing on the science of pharmacology. The action of a drug depends on the drug attaining a certain concentration in the blood stream and tissue fluids and a chemical reaction between the drug and the cells. Our knowledge of these chemical changes is as yet too limited to show how selective chemical changes are brought about upon a complex system. (Applied Pharmacology by A. J. Clark.)

Drugs may act independently outside the body (antiseptics on micro-organisms), they may act in or about the body, but not on its structures (sulphur on ringworm of skins) and lastly they may act on the tissues of the body itself. On the tissues of the body the drugs may act by physical or chemical means.

Physical and Chemical actions.

In physical actions no remarkable chemical reaction occurs between the drug and the tissues. The examples of physical action are the protective effect of oils, dusting powders, etc.; osmotic effects or salt actions of isotonic, hypotonic or hypertonic solutions; adsorptive or absorptive action of carbon dyes, infusorial earths and the colloids generally.

Some drugs exert chemical reactions within the body which may be of the nature of combination or substitution. By combination is meant the direct union

of the drug with the tissues of the body or with the products of secretion. Acids may unite with ammonia or with alkaloids to form addition compounds. Similar combinations may occur with protein. The action of general anæthetics is due to direct combination. Substitution occurs when the hydrochloric acid of the stomach is neutralised by sodium bicarbonate or the combination of calcium solution with oxalates to form inert insoluble compounds. Such unexplained reactions as selective affinity are probably chemical and may be either combination or substitution.

4. Selective or Specific Action

Specific Action.

Selectivity is a phenomenon widely distributed in nature. The eye selects certain vibration which are called light; other vibrations beyond the ordinary colour limit are unnoticed. The colour of objects is due to selective absorption. Foods and many drugs are readily absorbed from the gastro-intestinal tract, whereas most purgatives are only slightly absorbed. Carbon absorbs some gases and colouring matter to the exclusion of others. Platinum and palladium absorb some gases and not others. Selective oxidation occurs in the body, thus if 2.0 gm of benzene are given to a man 0.8 gm of phenol is formed, but if it is combined with 2.0 gm of alcohol, all the alcohol is oxidised and only 0.33 gm of phenol is formed. Selective actions in the body are apt to be modified by disease; sugar is readily oxidised in health but not in diabetes.

Mechanism of action.

The body cells show selective phenomena. The renal cells are very permeable to sulphates, the intestinal cells very slightly so. By virtue of this peculiarity the cells are capable of preserving their own composition notwithstanding the composition of the fluid in which they are bathed. This explains why a substance acts more strongly upon one cell than upon another. The differences are seen in case of the dyes. The difference in absorption may be due to the cell envelope and partly to the cell contents. Lipoid solvents generally penetrate better than other agents. The distribution of a drug is often modified by its reaction, or by the presence of a second substance, such as oxygen. Iodides and acridine dyes are taken readily by degenerating tissues, such as, caseous material.

Protoplasmic poisons.

Most drugs exert their action by their chemical affinity for one or other constituent of protoplasm. The living cell may be considered as a complex laboratory, where chemical decomposition, synthesis, reduction and oxidation are constantly going on. Drugs probably act by altering these processes so that either the functional power of the cell or actual cell structure is changed. Some of these are general in their action, minute quantities affecting practically all forms of protoplasm in the body (though not all forms in the same degree) and when the action of drugs is powerful they are known as general protoplasmic poisons, *e.g.*, hydrocyanic acid, quinine, etc. Some exert a selective action on a certain special tissue or tissues leaving the others unaffected. This is presumably owing to a chemical affinity for some component of the particular cells or group of cells. These are said to have a selective specific action. Strychnine has selective affinity for certain portions of the central nervous system, pilocarpine for secretory nerve endings, epinephrine for the sympathetic endings, atropine for parasympathetic nerve ending, picrotoxin for the medulla, etc.

Chemical nature of action.

It is suggested that during the production of specific effect the active drug is in some sort of combination with a chemical substance contained in the body of the cell acted upon and it is generally assumed that this combination is of a chemical nature. Adrenalin in producing its effect, is taken up and destroyed

by the sympathetic nerve endings; strychnine forms a loose combination with the cells of the cord for it is all recovered from the urine unchanged, morphine comes midway between adrenaline and strychnine as it is partly destroyed in the tissues and if the animal is tolerant, destruction is much greater than normal. The action of colloids may be selective. Other cases of selection may be explained on a physical or electrical basis.

5. Administration of Drugs

Frequency of administration.

As a matter of convention patients are used to take their medicines two, three or four times a day. Scientifically there is no such rigid rule, as the usual aim in therapeutics is to maintain the concentration of the drug in the body at an effective level. As different drugs vary widely in their absorption and excretion rates, it is obvious that there can be no uniformity in the rate of their administration. Quinine and other similar compounds, which are rapidly absorbed and rapidly excreted, have to be given at frequent intervals. On the other hand there are drugs like thyroxin which produce their action rather slowly (action of thyroxin comes into full play several days after its administration and lasts for about two weeks). In the case of such drugs it is not necessary to give the drug repeatedly during the twenty-four hours, as what matters is the total amount of drug taken during a number of days. Digitalis is another slow acting drug and here again there is no advantage in giving it two or three times a day. It can just as well be given in one effective dose every day to maintain its action. In the case of cumulative drugs (*e.g.*, digitalis) the initial dose required to bring the drug concentration in the body to an effective level is greater than the maintenance dose and care should be taken not to overdose the patient.

6. The Nature and Results of Drug Action

The fundamental properties of living matter as compared with dead matter are:—(1) Metabolism, *i.e.*, functioning of anabolic and katabolic processes. This includes nutrition or assimilation, which includes digestion, secretion, absorption and excretion. (2) Excitability as manifested by contractility, conduction, irritability and secretion. (3) Reproduction.

Drugs can only modify these properties, they cannot create new functions. The changes produced by the drug are quantitative only. The smallest live unit is a cell and the body tissues are made up of infinite number of these cells. The effect of drugs on cells is to stimulate them, to depress them or to change or destroy them. Stimulation is an effect on cells by which their power or their readiness to function is increased. It is often impossible to separate stimulation and sensitisation. Normally all movements are increased by stimuli acting on nerve centres. Depression is a decrease in function due in most cases to decreased sensitivity. In irritation the change is more anatomical than functional, and some of the signs of inflammation (redness, rise of temperature, swelling and pain) are present. Drugs may cause fatigue and paralysis which are also modifications of function. The causes of fatigue may be:—(1) exhaustion of energy-yielding material, (2) accumulation of waste products. Fatigue occurs in the following order in tissues:—nerve centres, nerve endings, muscles and nerve fibres. Recovery from fatigue takes place with rest alone. Paralysis may be produced by drugs and is due to a combination (chemical) of the drug with the cell substance; recovery takes place only after the drug is removed. Paralysis may also be caused by anatomical changes or lesions and in some cases recovery cannot be expected. In many cases fatigue and paralysis cannot be distinguished and in such cases both the elements usually enter into the action. Often a drug is

Stimulation and Depression.

found to stimulate one structure and depress another, *e.g.*, atropine stimulates the vagus centre and depresses the vagus endings; pilocarpine stimulates the nerve endings in sweat glands and depresses heart muscle.

Intensity of action.

The intensity of the action of a drug depends on, (*a*) the concentration in which it reaches the particular cells on which it acts and, (*b*) the duration for which this concentration is maintained. The concentration which a drug attains in the body depends on:—(1) The rate of its absorption, and (2) the rate of its removal from the body which occurs in two ways:—(*a*) Excretion by kidneys, lungs, skin, etc. (*b*) Chemical destruction in the body by processes of oxidation or reduction, or by the formation of inert bodies in combination with such compounds as glycuronic acid, glycocoll and sulphates.

7. Absorption and Distribution of Drugs

The local action of drugs occurs irrespective of absorption but for general action, absorption is necessary. There may, however, be local, general and specific actions from the same substance, *e.g.*, phenol. In many cases the object of administration is that they may be absorbed into the blood and produce their specific effects. For this purpose a drug may be (1) applied to the skin, but here absorption takes place with difficulty, as it has to be taken up through the sweat glands, the horny epithelium of the skin being impermeable; (2) when administered by the mouth the drugs are absorbed from the gastro-intestinal tract; (3) when injected subcutaneously, intramuscularly, intravenously or into one of the serous sacs absorption takes place rapidly.

Oral administration.

When given by the mouth, which is the commonest mode of administration, drugs are absorbed principally by the upper portion of the small intestine; very little absorption occurs from the stomach in case of most of the drugs. Experiments have shown that the isolated rectum absorbs certain substances at least as well as the small intestines, owing to its abundant vascularity. Strychnine 1½ grain put directly into the stomach of an animal produces convulsions in 30 minutes, in the small intestines in 10 minutes, in the œsophagus in 50 minutes, in the colon in 14 minutes and in the rectum in 7 minutes. From the stomach most drugs are absorbed slowly under ordinary conditions. Alcohol is, however, an exception and is not only absorbed rapidly but accelerates absorption of other substances dissolved in it, because its irritating effect improves the circulation. Such effects are reversible and the absorption of alcohol may be slowed by non-absorbable drugs such as cascara. Substances such as oleoresin of male fern are absorbed more quickly from solutions in oil than in water.

Absorption per rectum.

Factors regulating absorption.

Absorption from both the stomach and intestine is influenced considerably by the amount of food present and in some cases the food enters into combination with the drug. Absorption is more rapid from the empty than from the full gastro-intestinal tract. Absorption occurs readily from the small and more slowly from the large intestines. Foodstuffs that are not absorbed from the small intestines, are often absorbed from the large gut. In medical practice rectal feeding has sometimes to be resorted to in persons unable to swallow; predigested foods and glucose are readily taken up. Toxins are also absorbed in the same way. The rate of absorption depends on the method of administration of a drug and occurs in the following order beginning with the most rapid:—intravenous, intraperitoneal, intramuscular, hypodermic, through mucous membranes of sublingual region, nose, small intestines, stomach, rectum and from skin. Conditions affecting the alimentary canal also modify absorption, thus in shock absorption is considerably decreased. Slight irritation of the gut which does not cause injury may facilitate absorption. Pronounced injury may decrease it;

ulceration of the mucous membrane increases the absorbability of many drugs. Astringents tend to lessen absorption, and saline solutions such as magnesium sulphate and other cathartic drugs, which are but little absorbed themselves, may prevent the absorption of other drugs and even water.

Selective absorption.

The mechanism by which the mucous membrane of the gut allows the ready absorption of some substances and inhibits that of the others is not understood. Why K or Cl ions are absorbed and SO_4 and Mg ions are not, is obscure. The intestinal epithelium undoubtedly must have some selective power. It should also be noted that the presence in the alimentary canal of nonabsorbable substances hinders absorption of others, *e.g.*, absorption of strychnine in the isolated piece of gut is delayed in presence of magnesium sulphate. Some gases such as hydrocyanic acid, phosgene, nitrous oxide, and ethylene are absorbed rapidly by the lungs, while ammonia is but little absorbed.

Solubility.

Only soluble substances can be absorbed, but it should be remembered that solubility is modified by chemical changes in the alimentary canal; insoluble substances are rendered soluble by the action of juices and are then taken up. Solid substances such as carbon, iron, etc., are taken up through the agency of phagocytes and may be found in the mesenteric blood and lymph nodes. Such solids exert no action unless dissolved in the body fluids. The rate of absorption is modified by the solvent. Solutions in alcohol are more rapidly absorbed than water solutions. The more soluble a substance is in protoplasm, the more quickly it is absorbed. The concentration of the solution may vary the rate of absorption. The absorption area modifies the rate of absorption, *e.g.*, multiple hypodermic injections are absorbed much more quickly than if the same amount of fluid were injected in one place at once.

Volatility.

Volatility is another factor which influences absorption. Hydrocyanic acid is absorbed rapidly and causes instantaneous death when concentrated. Most volatile substances if swallowed are rapidly absorbed from the gastro-intestinal tract. Colloids such as gums and resins, oils, kaolin (Fuller's earth) charcoal and other inert powders and plant residues, when mixed with absorbable materials such as salts and alkaloids, lessen the rate of absorption—partly by fixing themselves to the drug and partly by hindering access to the absorbing surface. Isolated active substances (alkaloids) are, therefore, preferred if quick systemic action is desired, while galenical preparations (extracts, tinctures, pills, etc.), are generally used when there is no urgency. No general action may take place if the excretion of a drug is as rapid as its absorption, because the effective concentration cannot be maintained.

Rate of absorption.

Rapidity of absorption is proportional to the rapidity of the circulation and flow of lymph through the part. In cases of heart disease where there is stasis in the circulation of the intestine, digitalis may be poorly absorbed, and this accounts for the occasional failure of digitalis therapy in these conditions. For the same reason lesions of the spinal cord delay absorption. Vasoconstriction and catarrhal conditions of the intestines may also reduce absorption. Excessive distension of the gut decreases the rate of absorption by slowing the blood and lymph flow. Although drugs such as arsenic may travel for a considerable distance through dead bodies and strychnine, morphine, fuchsin, epinephrine, etc., have been shown to be absorbed in frogs with the heart removed, absorption normally takes place only when the circulation is intact. Deficient circulation delays absorption; some absorption however occurs through osmosis and diffusion even in the absence of circulation. In the alimentary canal, injury to absorbing cells may either facilitate or hinder absorption.

8. Absorption by the Blood and Lymph

Most soluble substances are absorbed from the alimentary canal by the blood rather than by the lymph and this is also true of serous cavities. Substances such as methylene blue, methanamine and some other drugs injected into the pleural sacs appear in urine before they are seen in the thoracic duct. All these drugs, however, may also be detected in the lymph. Inanimate particulate matter as well as bacteria, in their passage from the tissues to the blood stream go *via* the lymph channels and not the blood capillaries. Even from very recent wounds, which imply opened blood vessels, the above mode of travel holds good. Only those substances carrying a molecular weight of less than 5,000 (*e.g.*, cobra venom, strychnine) are taken up directly by the blood capillaries. Russel viper venom, black tiger snake venom, diphtheria and tetanus toxins all possess a molecular weight of over 20,000 and are thus carried to the blood stream by lymphatics. Recent work seems to have thrown some doubt on the assumption that some toxins can travel up through nerve trunks.

Molecular weight and absorption.

Non-absorbable Drugs.

A large number of drugs are either not absorbed at all or only in minute amounts. Of the common metals only arsenic and mercury are readily taken up and both of these are volatile; most of the heavy metals are absorbed so slowly that weeks or months of ingestion may be required to produce poisonous effects. Injection into the circulation of the same amount of iron, as given by mouth, may be attended with serious results. The difference in toxicity here is largely a question of absorption.

Absorption from Skin.

Absorption through the skin in man is very slow, though some substances such as methyl salicylate may be rapidly absorbed. The skin of young children is more permeable and death has occurred by application of methyl salicylate in this way. Absorption occurs more rapidly from regions where the epidermis is thin, *e.g.*, axillae, groins, inner surface of arms and thighs. That is the reason why inunctions are given in these regions. Potent drugs such as atropine and aconite, should be applied with care. If absorption is required from the skin, the drug should be incorporated with lanoline or lard. Lanoline is said to carry the drug to the tissues, lard to the skin only and petroleum to the surface of the skin. Absorption occurs quickly from the pleural and peritoneal cavities. Hypertonic salt solutions first draw water from the blood and when they are isotonic they are rapidly absorbed.

Factors regulating absorption.

Absorption is delayed by emotional states; fear, pain and sorrow may seriously interfere with the emptying of the stomach, digestion and absorption. Many pathologic conditions delay absorption. Absorption of fat is delayed in tuberculosis of the intestines on account of destruction of the lymphatics. In extraintestinal tuberculosis the absorption of all types of food materials is very considerably reduced.

Some drugs produce their effect because they are not absorbed, *e.g.*, some purgatives. The duration of action of a drug is limited by the rate of its excretion, its fixation by tissues, and by its detoxication by such processes as oxidation, reduction or combination to form inert substances.

9. Sojourn of Drugs in the Blood

Passage into tissues.

A drug, once absorbed into circulation, stays in the blood only for a short time. The passage of the absorbed or injected substances from the blood into the tissues is a rapid process for diffusible substances and materially slower for colloids. Intracellular absorption is a very rapid process again and depends

on the nature of the drug and to some degree on its concentration. Toxic doses of arsenic, after intravenous injection, disappear completely from the blood in less than 30 seconds, diphtheria toxins take 4 minutes, cyanides 2—6 minutes; anti-toxins circulate for several hours. A group of dyes remain for longer periods in the plasma, another group are rapidly excreted by the urine; and a third group disappear from the plasma, but are not excreted by the kidneys.

10. Time Taken to Produce Action

This is often an important question. After administration by mouth, a drug may take anything from half to 2 or 3 hours or more before the action is apparent. After an intravenous injection maximum concentration in the blood is produced immediately and response may also be immediate. In other cases the drug has to be activated in the body before producing its effect as for example is the case with organic arsenicals. Reaction between the drugs and the body cells may take time and probably this is the reason why even after intravenous injections atropine or ergotoxin may take five to fifteen minutes before producing effect.

Delayed action.

Certain drug actions are produced only after the drug has been in the body for prolonged periods. Radio-active compounds are examples of this class, and may produce death after a latent period of many years. With thyroxin dosage and duration of action is an important factor, a single large dose may produce little deleterious effects but the same quantity given in divided doses over a number of days may produce death. In case of lead, a single large dose is harmless but repeated small doses produce poisoning.

A single dose of a drug may produce ill effects the consequences of which may not be seen for a long time. In case of mercury diethyl, for example, the effects of a single dose, in the form of insanity and death, may not appear till after latent period of a month during which time the patient shows no symptoms. In case of toxic plants such as, *Senecio latifolia,* when fed to cattle for a few days, the toxic effects may not be appareat till after a latent period of three months or more; the poison in the meantime probably damages the liver. In case of X-rays and radium, erythema or burns may appear only after some days.

11. Distribution of Drugs in the Body

Storage in tissues.

This is not a uniform process. Drugs accumulate particularly in certain cells, according to their permeability and physical and chemical affinities. These influence their action either by bringing them in contact with reactive tissues, or storing them in places where they may be inactive. Iodine is stored in the thyroid gland as iodothyrin; bones retain earthy metals and fluorides; heavy metals are deposited as loose organic compounds in the liver and the spleen; mercury forms a loose globulin compound; with arsenic a more stable nuclein combination occurs in the Liver, bone marrow, skin, etc.; chlorides, bromides and related ions accumulate in all organs but mainly in the skin and the blood. Little is known about the distribution of organic poisons, mainly for want of suitable assay methods.

12. Changes in Drugs during Absorption

The majority of drugs are altered in the body by processes of oxidation, reduction, hydration, dehydration or decomposition; by storage in certain organs, or combination with other substances, toxic drugs are rendered harmless. This

process of detoxication is of very great importance as it renders the continuous administration of drugs (in most cases in increasing doses) an absolute necessity. If it were not for this power of the organism to destroy and remove poisons and thereby to recover from its action, all therapeutic uses of drugs would have been an impossibility. On the other hand, the alteration of the drug by the body may result in the formation of more toxic substances (nitrites) or the drug may be rendered more effective therapeutically as is the case with pentavalent organic arsenicals. The digestive juices destroy some organic poisons by hydrolytic changes (glucosides, proteins and anti-toxins). They are also necessary to saponify and liberate the active constituents of insoluble esters (phenyl salicylates). The acidity of gastric juice is of importance in the solution of bases.

Detoxication by tissues.

The tissues play a part in decomposing toxic drugs. Strychnine, morphine and many other alkaloids are partly oxidised in the tissues and thus rendered inert. Many organic acids, alcohols and formaldehyde are detoxicated by oxidation. The organic compounds of metals (*e.g.,* cacodyles) only develop their metal action after oxidation. The thyroid gland is said to play an important part in destruction of poisons in the body and its excision increases the toxicity of many poisons. The liver is believed to be a great disintoxicating organ, acting partly by destroying poisons and particularly by storing them. It possesses greater power to destroy drugs than other organs though alkaloid destroying ferments are found in other tissues also in small quantities. That is the reason why the same dose is much less effective when given into one of the mesenteric veins than when given into the jugular vein. This has been shown to be the case with curare, strychnine, morphine, cocaine, narcotine, quinine, atropine and the metals. Perfusion of alkaloids, glucosides, toxins, barium, etc., through an excised liver decreases their toxicity. Perfusion through excised muscle also produces a certain amount of detoxication but this is weaker than that is produced by the liver. Excision of the spleen is said to increase the toxicity of most alkaloids, but not all. Phagocytes accumulate and detoxicate many poisons, especially the colloids. The serum of atropine resistant animals (rabbit) destroys atropine.

Detoxication by liver.

The liver as stated above defends the body against toxic substances absorbed from the gastro-intestinal tract. It can detoxicate many substances, and those which it cannot detoxicate it can fix, thus preventing them from producing deleterious action on the vital organs. A common example is fixation of arsenicals, in the process of which it may receive serious injury. The kidneys like liver, can also pick up, store, or excrete drugs. This defence mechanism is not confined to the liver and kidneys but is of varied character. In the blood itself certain esterases exist which are known to neutralise the action of such potent substances as acetylcholine or adrenalin. The body has the power to produce and maintain antitoxins which neutralise the action of poisons of protein nature. When the action of a drug is required for a limited period, such drugs are chosen as are excreted rapidly or are rapidly detoxicated in the body.

13. Adsorption

Adsorption comes from the Latin word '*Sorbere*' meaning to suck. It is the power possessed by certain substances of retaining on their surfaces gases, liquids, and solids, either in solution or in the colloidal state. One substance here becomes a part of another and remains in a state midway between mechanical mixture and chemical combination. This phenomenon plays a very important part in the action of drugs. The disintoxication is sometimes carried out through adsorption, thus pilocarpine is detoxicated in excised intestine by digestion with serum; but it may be fully recovered from it in active form by extraction with acid or alcohol, showing that it was not destroyed. The nature of adsorbing substances is not known, but it is found in abundance in rabbit's serum, less in that of cats or oxen, and it is absent in dog's blood.

14. Excretion of Drugs

Channels.

The main channels of excretion are the kidneys and bowels, and with volatile drugs the lungs. The sweat and for that matter other secretions play a comparatively minor part. The relative importance of different channels varies for each drug. The excretion is proportionate to the circulation of the blood and

may be increased by factors which stimulate it. The excretion of certain drugs is limited by their being in the body in the form of combinations; the elimination of these is favoured by substances which displace them in the compounds (excretion of iodides is increased by giving chlorides).

Certain drugs are excreted by sweat, *e.g.*, iodides, bromides, borates, phenol. salicylates, antipyrin, methylene blue, arsenic and mercury. The quantities thus got rid of are too small to be of any significance from the point of view of elimination, but they help to explain certain skin conditions which accompany their administration. Excretion by saliva is limited to iodides, potassium, ammonium, mercury, lead, menthol, guaicol and some alkaloids (morphine and quinine). The excretion generally begins within 20 minutes and lasts for nine hours.

15. Passage into Cerebro-spinal Fluid

Selective absorption.

The lining cells of the choroid plexus exercise selectivity in letting through various substances into the cerebro-spinal fluid. This is the reason for such differences in the concentrations of various normal chemical constituents in the plasma and the cerebro-spinal fluid. For a number of compounds such as the citrate and iodide of sodium, and also for colloids of the plasma, the choroid plexus lining is a complete barrier. Bile pigment also, even when present in the plasma in high concentrations do not penetrate into the cerebro-spinal fluid except in negligible quantities. Many substances on the other hand, pass from the serum into the cerebro-spinal fluid quite easily as for example, morphine, strychnine, pilocarpine, volatile anaesthetics, alcohol, bromides, salicylates and the basic dyes like toludine blue, pyronine, safranine and neutral red. Sulphonamides are the latest addition to this group of drugs, their concentration in the cerebrospinal fluid being about 75 per cent of that in the plasma.

As a general rule, passage of various substances from the blood to the cerebro-spinal fluid is regulated by the same laws which operate for the passage of the same compound from blood to brain tissues. This passage is related to electrical charge of the molecules; positive or neutral charges get through rapidly whereas negatively charged substances find their transit barred.

16. Passage to Fœtus

Chloroform, ether, alcohol, chloral, ethyl bromide, scopolamine, quinine, atropine, morphine, arsenic, mercury, potassium iodide, potassium bromide, carbon monoxide, salicylic and benzoic acid, phloridzin, nitrates, urea, methylene blue, pass into the fœtus if given to mother. The placenta acts as an ultra-filter towards colloids. The colloidal dyes and proteins do not pass into the fœtal circulation.

17. Toxic Effects

Definition of poison.

If drugs are given in adequate doses they produce therapeutic effects, but if larger doses are given they act as poisons. Poison comes from the Latin word *potio* meaning a draught. By poison is meant any substance, administration of which will injure health or cause disease. These effects may be immediately manifested or they may take some time to occur. Toxicology comes from the Greek word *toxikon* meaning a poison. It deals with the symptoms, diagnosis, treatment and detection of poisons. As a large number of the drugs used in the treatment of disease are potent substances, their dosage and the length of period during which they are to be administered should be very carefully considered. The safety factor in dosage is often assessed by considering the

Safety factor.

ratio of therapeutic dosage of the particular drug to its minimum lethal dose. As minimum lethal dose is not a stable definite figure it is preferable to base calculations on the average or the median toxic dose. The physician should be clear about the chances of a particular dosage producing toxic symptoms. As a rule it is not justifiable to use a dose which carries even a 1 in 1,000 chance of producing serious or fatal toxic effects. Even when the dose is considered safe and this is specially true of powerful remedies, the final adjustment in the dose should always be governed by the response elicited from the patient who should be under careful observation.

18. Chemical Constitution and Physiological Action

If action of drugs could be anticipated by the knowledge of their chemical formulae, the task of discovering effective remedies for diseases at present without specific cures would have been considerably eased. At present new remedies are hit upon mostly by mere coincidence and sometimes hundreds of compounds are turned over before one can be found which affords reasonable chances to merit a clinical trial.

Even very slight differences of chemical constitution in seemingly alike drugs may mean a material divergence in their action.

The laevo-rotatory isomer of epinephrine is seventeen times stronger than dextro-rotatory isomer. On the other hand drugs having a very dissimilar constitution may have similar actions. Drugs prepared for one action may produce action of a different kind altogether. The search for remedies is, therefore, a laborious process of trial and failure.

CHAPTER II

CONDITIONS MODIFYING THE ACTION OF DRUGS

DOSAGE—ACCUMULATION OF DRUGS—HABIT—SEX, RACE AND CLIMATE—TIME AND FREQUENCY OF ADMINISTRATION—IDIOSYNCRACY, SUSCEPTIBILITY AND TOLERANCE—INDIVIDUAL VARIATIONS IN THE ARSORPTION OF DRUGS FROM THE GASTROINTESTINAL TRACT—SYNERGISM AND ANTAGONISM—EXTERNAL FACTORS—STANDARDISATION AND BIOLOGICAL ASSAY.

1. Dosage

By dosage is meant the quantity of a drug required to produce action either immediately or after repetition. The largest quantity which can be given without untoward effects is called the *maximum dose*. The dose of a drug usually bears a direct relation to body weight and it is rational to calculate it per unit of body weight when full response, specially in case of powerful remedies, is being aimed at. However, in case of certain drugs there is no relation between the body weight and the dose required. In case of insulin, for instance, the dose is directly related to the severity of the disease and not the weight of the patient. Again in case of antitoxic sera their dose is governed by the amount of toxin requiring neutralization. Young persons require smaller doses than older ones, since younger tissues react more readily and also because the weight is less. In children the dose is usually given according to age. *Definition.*

Many practical rules have been devised for calculating the doses for children when the adult dose is known. *Dosage in Children.*

(*a*) Young's rule: $\text{adult dose} \times \frac{\text{age}}{\text{age}+12}$

(*b*) Cowling's rule: $\frac{\text{adult dose} \times \text{age at next birth-day}}{24}$

If prescribing 24 doses all that is required is to multiply the adult dose with age of the child in years.

(*c*) Bush's rule. Multiply the age by 5, this gives the percentage of the adult dose.

(*d*) Dilling's rule: $\text{adult dose} \times \frac{\text{age}}{20}$

In this connection Clark has formulated a method of dosage for a person of known weight— $\frac{\text{adult dose} \times \text{weight of person in pounds}}{150}$,

150 lb. is taken as average weight of an adult person. This method, though useful, is not always feasible as it is impossible to weigh a patient lying in bed. A rough estimate of the probable weight can, however, be made.

A single dose may be given or divided doses may be administered (1/4 gr calomel every half hour till 2 to 4 grains are taken). Repeated doses may be given (1) to have effect just at the time of administration, or (2) to have continuous effect (digitalis for disordered heart). A drug may have different effects according to the dose in which it is given, *e.g.*, ammonium carbonate is an expectorant in 3—10 gr doses and an emetic when 30 gr are given.

2. Accumulation of Drugs

The majority of drugs have to be given in repeated doses to get a sustained effect. In medical practice drugs are prescribed two or three times a day or oftener. Drugs which are rapidly excreted or detoxicated produce action for a limited time and by repetition this action can be constantly maintained. If, however, a drug is not removed as quickly as it is absorbed, its concentration will increase in the body and increasingly powerful effects may be produced which may be deleterious to the system. Such drugs are called cumulative poisons and digitalis is an excellent example. *Cumulative action.*

Accumulation is liable to occur also in case of metallic drugs which cannot be destroyed or detoxicated in the body and which are as a rule slowly excreted; mercury, lead, arsenic, bismuth, etc. are examples of such drugs. It may also occur when the excretion is decreased or suddenly stopped for some reason *e.g.*, when there is nephritis, or a sudden solution and absorption of a sparingly soluble drug owing to some change in intestinal content. Successive doses of the drug may remain unabsorbed in the alimentary canal,

in the muscle or subcutaneous tissue, to be finally taken into the system when the conditions are more favourable for absorption. It sometimes happens that effects are more easily reproduced after they have been once induced. This is particularly the case with drugs acting on the central nervous system. Susceptibility to strychnine increases with its administration and is said to be due to the central nervous system becoming educated to the stimulating actions and responding to them more.

3. Habits

Tolerance.

Habit usually lessens the effect of drugs to some extent; persons habitually using morphine or opium need bigger doses of these drugs; persons taking large doses of alcohol need larger doses of hypnotic and anaesthetic drugs. In a few cases the reaction is greater after continued use, as in case of the purgative cascara sagrada. In the case of habit-producing drugs tolerance may be largely psychic. The nervous centres learn to modify the reactions so as to adapt them to drug environments. When this environment is abruptly altered by withdrawal of the drug, there is generally extreme discomfort, resulting in craving and great nervousness, constituting abstinence symptoms. Functional habituation, when acquired for a particular drug, may hold also for other drugs having a similar action.

4. Sex, Race and Climate

Women are more delicate and lighter than men and need three-fourths to four-fifths of the dose of men. Idiosyncracy occurs more often in the female sex; during the menstural period certain drugs are not advisable (*e.g.,* quinine may cause excessive bleeding). Pregnancy and lactation should also be taken into consideration as some drugs are excreted in milk, while others have action on the gravid uterus.

Various species of the same family show remarkable difference in their reaction to drugs. Different organs of the same animal exhibit diverse reaction to drugs such as adrenalin, atropine, pilocarpine, etc. Racial differences may also play a part in the action of a given drug.

The climate in which a person lives renders him more or less susceptible to certain remedies, *e.g.,* alcohol is better borne in cold climates. The temperament of an individual is an important factor; phlegmatic dark-skinned people often react to drugs less readily than blondes and nervous patients, especially in respect of drugs which act on the nervous system.

5. Time and Frequency of Administration

This is of importance, *e.g.,* saline purgatives act most rapidly on an empty stomach after a period of fasting, so they are usually given in the morning before breakfast. The more slowly-acting purgatives are given at bed time and half an hour before meals. Irritant drugs, such as arsenic or iron, are best given after meals, when they become well diluted with the stomach contents and come in contact with the stomach wall in lesser concentration and do not irrate it; quinine sulphate is best given 1½ to 2 hours after meals, because it is more quickly absorbed when the contents of the stomach are acid and besides it is less liable to disturb digestion at this time; digitalis preparations are best given by themselves on an empty stomach. Soporific drugs are most effective at the natural time of sleep; they may be without effect if the patient is not in bed and is up and about. Sodium bicarbonnate is given on an empty stomach because, if given during the digestive period, it neutralizes the hydrochloric acid of the gastric juice.

Relation to meals.

Relationship between the time of administration of a drug and meal-time may influence not only the rate of absorption, but also may modify its action. Given on a full stomach, the absorption is slower than when given some hours after meals; if local action is desired on the stomach, the drug should be given before meals. Whenever possible, a purified standard preparation should be used.

Frequency of administration is important as drugs are rapidly oxidised, destroyed or stored in the tissues or eliminated through secretions or excretions. The rapidity of destruction determines the frequency with which they should be administered. The speed or rapidity of administering drugs may mean difference between life and death.

6. Idiosyncracy, Susceptibility and Tolerance

No two individuals respond to drugs in an identical manner. Drugs may exert an unexpected effect either by having an unusual action or failing to produce ordinary action The term idiosyncracy is applied to peculiar, exceptional reactions to the effect of drugs. Fortunately only a small minority of individuals react to certain drugs in an entirely abnormal manner. Acetylsalicylic acid, for example, in such individuals, may produce extensive urticarial eruption or even œdema of the mucous membrane of pharynx and

Abnormal reactions.

larynx, giving rise to serious symptoms. There may be quantitative hyper-susceptibility to ordinary actions of drugs, so that side actions, which are ordinarily negligible, become greatly accentuated. Neurotic individuals are subject to psychic exaggeration or modification of drug effects through fright, excitement or suggestion. They are frequent causes of disappointment both to the physician and the patient and may give rise to excessive action from what has been thought to be a moderate dose.

Idiosyncracy.

Allergic hyper-susceptibility is a type of idiosyncracy in which the patient reacts to special substances, food or drugs, by irritant œdema and spasm of smooth muscles, urticaria, bronchial asthma and collapse. It is generally hereditary but not congenital, *i.e.*, it may manifest itself in later life. It resides in the tissues so that it cannot be transferred to the blood serum. It can be induced in excised tissues by treatment with colloidal precipitants. Some people develop a rash or even very serious symptoms after eating strawberries, others after taking certain kinds of fish. Sometimes all members of a family show such an idiosyncracy to a special article of food or to a particular drug. Thus it has been reported that a minute amount of cocaine dropped into the eye or a few grains of quinine taken internally have produced very serious symptoms. The cause of this condition is not quite clear. Slight alteration in the composition of tissues may cause similar conditions, *e.g.*, deficiency of calcium renders the whole autonomic nervous system more susceptible to the action of certain drugs. The variations in reactions of different animals or racial idiosyncracy can be explained by difference in physiologic functions; for instance, cerebral reactions are usually predominant when the central nervous system is highly developed, while spinal reactions predominate in lower vertebrates. Rabbits are incapable of vomiting, therefore, they cannot be affected by emetics. Normal resistance may be due to destruction of the poison, *e.g.*, atropine by serum of rabbits. Differences of absorption and excretion also come into play. There are many factors, however, which cannot be satisfactorily explained. It should not be forgotten that abnormal reactions following the administration of drugs may rarely be due to the presence of impurities.

Tolerance.

Some animals and men fail to react to drugs even when given in considerable doses and this phenomenon is known as tolefance which may be natural or acquired, *i.e.*, developed by repeated administration of a drug. Natural tolerance may be due to the power of the tissues to neutralise poisons. Thus carnivora can stand large amounts of acids owing to the production of ammonia in their tissues; chickens are immune to oxalic acid when given by mouth, because of the large amount of calcium in their intestines. The susceptibility of the cells to a drug varies enormously in animals. For example digitalis glucosides have a powerful effect on frog's heart but no action on that of a toad.

Mechanism.

Cellular immunity can undoubtedly be acquired in case of a large number of drugs e.g. alcohol, morphine, arsenic, etc. But according to Clark it is probably due to some mechanism which prevents the drug from reaching the susceptible tissues in high concentrations rather than due to cellular immunity.

Tolerance versus immunity.

Acquired tolerance has already been considered under HABIT. Tolerance is usually limited, not absolute. Any condition which lowers the general resistance of the animal increases its susceptibility to the poison (frog in tepid water). Tolerance differs from immunity in that anti-toxins are said to be formed in the latter. Anti-toxin formation, however, is an instance of acquired tolerance. It was supposed to be confined to proteins, but it appears to exist also towards certain glucosides (toad-stool and snake venom). It does not occur with alkaloids. It is doubtful if any chemical substances except proteins produce anti-bodies.

It will thus be seen that methods by which the body can protect itself against drugs are varied and that tolerance is a result of complicated factors.

Drug Addiction is a separate problem but is dependent on tolerance. Tolerance may, however, be produced quite independently of addiction. The subject has been dealt with in detail later.

7. Individual Variation in the Absorption of Drugs from the Gastro-intestinal Tract

Physiological considerations.

In order to understand individual variations in the absorption of drugs, it is essential to appreciate the digestive functions of different parts of the alimentary tract, because absorption of drugs is intimately connected with the digestive processes. From the point of view of absorption the alimentary tract has been divided into three main sections. These divisions are not anatomical but purely from considerations of function. The upper portion comprises the mouth, the stomach and the upper part of the duodenum. Its functions are to receive food, to reject unsuitable material, to reduce the remainder to a fairly

uniform consistency by chemical and mechanical means. The digestion of food is started here, and the contents are passed on to the middle portion.

The middle portion begins in the lower part of the duodenum and extends to the ileo-cæcal sphincter. Its functions are to complete the digestion of food and to absorb as much as possible. Nearly 90 per cent of the absorbable substances in a meal are taken up here.

The lower portion consists of the cæcum, the colon and the rectum. Its functions are to complete absorption, to store and to periodically excrete the indigestible residue. The contents of the small intestine enter this protion in a fluid state, and are reduced to a semisolid consistency. Certain reflexes are set up periodically and the contents are expelled. Absorption from the rectum and colon is small, and their utility from the point of view of nutrition or medication is very limited.

The main factors concerned in individual variability in the absorption of drugs may be grouped under three headings:—

(*a*) *Variations because of physiological factors.*—The most ancient, the most natural and even now the most widely used method of administration of drugs is by the oral route. Its chief advantages are undoubtedly from the point of view of the patients who can make use of medicaments without the intervention of the doctor or any other person. A majority of the drugs given by mouth reach the stomach without much alterations on account of rapid passage through the mouth, the pharynx and the œsophagus. No appreciable absorption takes place even from the fundus of the stomach. It may be stated here that in case of some drugs considerable absorption may occur from the mucous membrane of the mouth, and this method is adopted in case of some diffusible drugs (*e.g.*, nitro-glycerine, adrenaline, some sex hormone preparations), but this cannot be regarded as a suitable method for most of the other drugs in use. It is only when a drug passes through the pyloric sphincter that the absorptive processes really begin. The activity of the stomach, therefore, plays an important part in the commencement of absorption because it is the muscular contractions of this organ, which push the contents through the pyloric sphincter after they have been dealt with by the gastric juice. The passage of a drug through the stomach may thus take from a fraction of a minute to more than two hours.

Physiological factors.

Effect of stomach contents.

If a non-irritant drug is swallowed when the stomach is empty, and, when a large quantity of water is taken with it, it rapidly passes through the stomach into the duodenum and absorption commences immediately. If, however, a drug is taken after a meal, its absorption is very variable. For instance, if a non-irritant drug is taken with a large amount of fluid, it may pass along the lesser curvature of the stomach straight to the pylorus and through it into the duodenum. If the volume of fluid is small, the drug will mix with the food mass, pass slowly through the pylorus with the food and absorption will be considerably delayed. Absorption of a drug from an empty stomach is often irregular specially if it is irritant, as it may reflexly cause closure of the sphincter and thus prevent its reaching the absorptive surface of the duodenum.

Effect of circulation.

Circulation of blood in the gastro-intestinal tract has considerable influence on absorption. Absorption is quick when the circulation is normal; it is slowed when it is hampered by such factors as vaso-constriction, catarrhal conditions of the mucous membrane, etc. If the physiological processes regulating the activity of the gastro-intestinal tract are borne in mind, it would be easy to insure either slow or rapid absorption within certain limits.

Physical and Chemical factors.

(*b*) *Variability because of physical and chemical factors concerned.*—The phenomena of solution, filtration, osmosis, diffusion, dialysis, and phagocytosis are all actively concerned in the absorption of drugs. In addition to these, a mechanism which is not understood occasionally comes into play. This has been termed the 'vital activity' of the cells of the mucous membrane, and defies all the physical and chemical laws and endows the cells of the mucous membrane concerned with special powers of selection or rejection of the material at its disposal. Absorption of the animal's own serum, for example, takes place from the intestinal canal without alteration whereas other sera undergo normal protein digestion.

Effect of solvents used.

The rate of absorption of a drug is modified by the solvent used. For instance, alcoholic solutions are more rapidly absorbed than aqueous solutions, probably because of the greater diffusibility of the solvent. The more soluble a substance is in the protoplasm the more quickly it is absorbed, *e.g.* glucose, urea. Colloidal substances, such as gums, resins, oils, kaolin, etc., are absorbed with difficulty; in fact, when absorbable materials (*e.g.* salts, alkaloids, etc.) are mixed with such substances, the rate of their absorption may be so much slowed that no action occurs, the rate of excretion being as rapid as absorption.

The concentration of solutions affects the rate of absorption in conformity with the laws of osmotic pressure. Saline solutions such as magnesium sulphate which are little absorbed themselves may prevent the absorption of readily absorbable drugs. Astringent substances tend to lessen absorption. Solids are not suitably altered by the intestinal juices and are not absorbed except through the phagocytes; they may even then be deposited in the lymphatic tissues. They exert no action unless dissolved by body fluids.

Effect of disease.

(*c*) *Variation in absorption due to disease.*—When the normal functions of the stomach are deranged on account of disease, considerable changes occur in the absorption of drugs. Thus in case of shock absorption is considerably decreased and may even stop altogether. Slight irritation of the gut, which does not cause injury, increases absorption, but absorption is decreased and may be totally inhibited when the gut is injured.

In febrile states, it would not be safe to estimate the probable rate of absorption. In a sick person the rate of absorption may differ considerably from that which occurs in health. Often absorption is considerably reduced when there is general reduction of vitality; the lower resisting power of the body occuring in chronic constitutional diseases retards absorption. If the intestines themselves are diseased they allow much more rapid diffusion of proteins, toxins and ferments than healthy intestines but absorption of drugs may be adversely affected.

Effect of gastro-intestinal lesions.

It is not surprising, therefore, that the same drug given to two different patients under similar conditions for the same symptoms, may behave entirely differently, and on testing the urine, one patient may show the presence of the drug and the other may not. The reason of this variation is not far to seek. An analysis of the records of patients admitted into the Carmichael Hospital for Tropical Diseases, Calcutta, showed that in as many as 60 to 70 per cent of patients the gastro-intestinal tract was involved. Detailed investigations revealed that as many as 25 to 30 per cent suffered from helminthic infections, 28 to 30 per cent from catarrhal conditions of the intestines of various origin. The remaining suffered from pathological appendix, gastro-duodenal ulcer, diseased gall-bladder, etc. Helminthic parasites especially hookworm live in the upper part of the small intestine, and even a light infection may alter the normal physiological processes in this part.

Helminthic infestations.

In addition large number of patients complained of vague abdominal symptoms although no definite laboratory finding for known infective agents was obtained. Most of these patients showed a condition of hypo- or hyperchlorhydria which is very commonly associated with chronic dysenteries and colitis in which the original causal agent, either bacterial or protozoal, is very difficult to detect. The acidity of the stomach, which is often altered in these conditions, is in itself a factor in the absorption of the drug inasmuch as it is often here that the drug is rendered suitable for absorption by disintegration of tablets and conversion of insoluble into soluble compounds.

Dysenteries and diarrhoeas.

Another important factor which should not be lost sight of is that the rapid passage of a drug through the small intestine, where absorption usually takes place, occurs in chronic diarrhœas and dysenteric conditions. *X*-ray examinations with barium meal show that the meal in these cases hurriedly passes through the small intestine, and in a very few hours, less than five usually, it reaches the pelvic colon, no trace being left in the small intestine. Drugs therefore are not allowed sufficient time for absorption. Besides these conditions, the mucous membrane of the gastro-intestinal tract is not normal in a large percentage of patients in the tropics, and peptic ulcer, disease of the liver and gall-bladder and pathological appendix, colitis, etc., are frequently met with. The absorption of drugs in such cases may be far from normal and may show considerable individual variations.

The importance of these factors was emphasised during clinical trials of different anti-malarial drugs. Results with even such potent drugs as plasmochin and atebrin, were so variable, that it was not till the excretion of these drugs in the urine was studied side by side with their effect on the malarial parasites, and clinical symptoms in a large series of cases, that the cause of these discrepensies was understood. It was found that in some cases. the absorption of such drugs as quinine and atebrin, was considerably delayed, in other cases it was very small and yet in other cases, there was no absorption at all. What is true of atebrin and plasmochin holds good in case of other drugs also. Every medical practitioner is familiar with the wide individual variations in response to drugs. No two individuals respond to any drug in an identical manner, and a small minority may react to certain drugs in an wholly abnormal manner. Extensive data in this connection has been gathered from biological standardization of drugs with different stocks of animals and large variations in dosage and response are found. If this is the case with healthy animals, it is not surprising that large

variations occur in disease and particularly when the disease affects the gastro-intestinal tract. The physician should carefully consider absorption and excretion of drugs before administering them and when expected results are not forthcoming. From observations entertained over many years by the author, the following factors were found to be responsible for variability in the absorption of drugs from the gastro-intestinal tract. The practitioner in tropical and sub-tropical climates should bear these in mind:—

1. Absorption of drugs is considerably modified in acute and chronic inflammatory conditions of the mucous membrane of the gut.

Factor affecting absorption.

2. Hypoacidity of the stomach which is often met with in chronic dysenteric and other infections has considerable effect over the absorption of drugs.

3. Rapid passage of meals through the small intestine generally, and through its upper part particularly where these drugs are usually absorbed, hinders absorption. This is quite commonly met with in amœbic and bacterial dysenteries and affections of the liver.

4. Drugs administered in form of tablets which may not be properly constituted. Some of the ingredients used may prevent their disintegration before reaching the part where absorption of drugs takes place. In some cases the tablets may not disintegrate at all, and may pass through the gut unchanged.

8. Synergism and Antagonism

Synergism.

A drug exerts its usual activity much more easily if it is given with some other drug of the same class, and it is found that combination of two drugs having the same action will give a result which one alone will not produce, however large the dose might be. Such drugs are called synergists or mutual helpers and the phenomenon is known as synergism. In some cases new actions develop by the reaction of drugs with each other, with the production of new compounds (acid renders the basic salts of bismuth soluble and toxic).

Combination therapy.

Synergism of drugs was responsible for combination therapy which had the idea of securing summation of the desirable effects of several drugs; the side actions usually do not increase and in some cases they are neutralised. Formerly this form of therapy was popular and shot-gun prescriptions were given with the idea that some at least of the many ingredients might attack the disease. Such indiscriminate empirical use of drugs, however, is not scientific and it is better to employ only a few drugs with known actions. More recentlly. the combination method has been tried with great success in treatment of protozoal diseases, for this is the principle underlying chemotherapy. In combining several drugs having a similar action the dose of each should be correspondingly reduced.

Summation effects.

The best examples of synergism are seen in the group of narcotics. In the case of alcohol, ether and chloroform the effect is simple summation, but in case of morphine and scopolamine there is considerable angmentation of effects. Again an injection of magnesium sulphate considerably helps the action of ether in the production of general anaesthesia. A mixture of purgatives, *e.g.,* calomel, jalap and colocynth, act better than any one of them alone.

The synergism may be produced by one drug modifying the penetration of the other into the cell, altering the chemical affinity or attacking the cell from a different point of view. Magnesium potentiates urethane, and ether potentiates chloral or morphine, by favouring their distribution in the central nervous system.

Antagonism.

On the other hand, a drug may lose a part or all of its action because of some agent which has the opposite physiologic effect. Such opposing agents are called antagonists and the phenomenon is known as antagonism. There are examples of antagonistic substances being manufactured by the body itself (insulin and adrenalin). The antagonism between drugs and poisons when this depends on the antagonistic innervation of certain organs is easy to understand (*i.e.,* vasodilators and vasoconstrictors). Much more difficult to understand is the antagonism by which one drug overcomes the effect of another in the same cell without the aid of physiological mechanism. This may be brought about in two ways:—

Mechanism.

(1) By chemical changes or combination as in case of free acids and alkaline carbonates, oxalates and lime salts.

(2) By true antagonism, as in case of atropine and muscarine, which is of truly physiologic nature, for these two drugs have no chemical affinities for each other, but produce directly opposite effects on the same organic element. The action of potassium chloride and ammonium chloride on the yeast ferment invertin, gives a simple type of this

antagonism, the first inhibiting and the latter favouring, and the two together in proper proportions leaving the activity unaltered. In other words, there is complete reciprocal antagonism. The possibility of this has been denied on the ground that while it is possible to bring about paralysis in a stimulated organism, it is not possible to bring about stimulation in a paralysed one. It must not be forgotten, however, that combination between poison and protoplasm is of a dissoluble nature, so that cells may be restored again and poison washed out from them if bathed with blood which is free from the poison. Reciprocal antagonism between calcium and magnesium and sodium and potassium is clearly demonstrated in living organism, for the tissues can maintain their normal function, particularly their normal excitability, only when these kations are present in their correct relative proportions. *Reciprocal antagonism.*

Antagonistic drugs may act, (*a*) on the same structures, *e.g.*, bromide and strychnine on the spinal cord; caffeine and alcohol on psychic and motor centres in the cerebrum; pilocarpine and atropine on the vagus nerve-endings; (*b*) on different structures, *e.g.*, digitalis slows the heart by stimulating the vagus centre, atropine prevents this by depressing the vagus nerve endings. Adrenalin contracts vessels by stimulating the vaso-constrictor fibres while nitrites relax them by acting on the muscle fibres.

9. External Factors

Some drugs are more potent in the presence of ultraviolet rays than in dark. Temperature is an important factor, the effect of most drugs being more intense at higher than at lower temperatures. Atmospheric pressure is another factor; thus carbon monoxide does not kill if *operating under a pressure of* several atmospheres. Bacteria and enzymes may also play an important role.

10. Standardisation and Biological Assay

In order to obtain uniformity in strength of preparations, the British and the United States Pharmacopoeias have laid down methods of chemical and biological assay. This secures means of measuring and furnishing uniform preparations and in this way their therapeutic activity is safeguarded. Drugs having active principles of known composition can be standardised by methods of chemical assay which have been laid down in case of most of the preparations. Physical assays are carried out in form of saponification values, refractive indices, optical rotation, specific gravity, iodine values, and melting points. *Chemical and physical assay.*

In case of a number of drugs, however, the active principles are either unknown or are of such a complicated nature that ordinary methods of chemical assay are not applicable. Such drugs are assayed by biological methods. The principle underlying is to estimate the strength of the preparations by determining the minimum lethal dose to animals under standard conditions of weight and species. In other cases the standardisation is carried out by noting the physiological effect produced on some organs, such as heart, uterus, ovaries, etc. According to the British Pharmacopœia the following preparations are assayed by biological methods:— *Biological assay.*

Neoarsphenamine and sulpharsphenamine, digitalis (powder and tincture), strophanthus (tincture), strophanthin, insulin, pituitary (posterior lobe) extract, old tuberculin, diphtheria antitoxin, gasgangrene antitoxin, anti-dysenteric serum, tetanus antitoxin, staphylococcus antitoxin, anti-pneumococcus serum, vitamins A, B, C and D.

CHAPTER III

MODES OF ADMINISTRATION

INTRODUCTION—LOCAL APPLICATION: ON SKIN, ON MUCOUS MEMBRANES, IONIC MEDICATION—ORAL AND RECTAL ADMINISTRATION—ADMINISTRATION OF DRUGS BY INJECTION: SUBCUTANEOUS OR HYPODERMIC ROUTE, INTRADERMAL ROUTE, INTRAMUSCULAR ROUTE, INTRAVENOUS ROUTE, OTHER ROUTES.

1. Introduction

By mode of administration is meant the way in which a remedy is to be used. Remedies are given to obtain either direct local action, a remote local action, a systemic or general action.

Local action.

The direct **local** action of a drug is exerted at the place at which a drug is applied, *e.g.*, skin, nose, **urethra,** etc. Locally, drugs may act by protection, irritation, or depression of sensory nerve endings or oher tissues. Many drugs act locally only, others like strychnine, exert little or no effect at the point of application, but exert a marked general effect. Local remedies may or may not require to be absorbed, *e.g.*, bismuth subnitrate for an irritated stomach, talc powder for skin which is chafed.

Remote action.

Remote or indirect action is an action elicited on organs away from the site of application. Such action of drugs may appear (*a*) after its absorption into the circulation, *e.g.*, of strychnine on the spinal cord; pilocarpine on the secretion; (*b*) without actual absorption, *e.g.*, irritation of the skin, blisters or cold applications influence the rate of the heart, indirectly through the central nervous system. Sometimes the effect of a drug is manifested as it is being excreted, *e.g.*, irritation of the bowels and kidneys by perchloride of mercury as it is passed out *via* the colon and the urine; antiseptic action of urotropine as it is eliminated in the urine.

General action.

The general action is one that cannot be fixed in any particular tissue, *i.e.*, the action of many tonics and sedatives.

The effect of a remedy depends largely on the way in which it is given, *i.e.*, adrenaline given by month has a local action on the stomach only, while if given hypodermically or intravenously it produces an enormous rise of blood pressure. A dose of saponin by the mouth is perfectly harmless, but it gives rise to poisonous symptoms if given under the skin. Some tissues and organs absorb much more rapidly than others, therefore, larger quantities of the drugs, pass through them into the blood in a given time. For instance, if a poison which is absorbed slowly, be rapidly excreted, so little of it will exist in the blood and tissues at a time that hardly any action is produced; while if it is rapidly absorbed by some other method of administration, even a small dose will exert some action before it is excreted, *e.g.*, potassium salts given by the mouth are not poisonous in large quantities, but when given hypodermically or intravenously they produce toxic effects on certain organs.

The channel by which a drug is introduced into the body or the place to which it is applied, must necessarily vary with the object to be secured, *i.e.*, whether the action is to be purely local or general; whether rapidity of absorption is desired or whether there is any necessity of avoiding irritation to certain organs, etc.

2. Local Application

Locally, drugs may be used to protect surfaces, for producing reflex effects, for antiseptic or stimulant purposes.

(1) On Skin

Epidermal absorption.

On the skin, drugs may be applied in various vehicles. If it is desired the drug should penetrate deeply or be absorbed it should be mixed with animal or vegetable fats. The reason is that although the lower layers of skin absorb readily, the stratum corneum of the epidermis is non-permeable to most substances. The absorption takes place through the glandular structures which are filled with fatty matter. Increased vascularity of the skin favours absorption by carrying away quickly the absorbed drug. Where local effect alone is desired, mineral fats (vaseline) may be used. Oils and ointments delay the absorption of fat-soluble substances, such as phenol, mustard oil, etc.

Local application to the skin has to be used for producing general effects in those cases where the stomach must be avoided and the subcutaneous method is not practicable, *e.g.*, inunction of mercury. The objection to this method is that (1) absorption is uncertain and consequently exact dosage is impossible, (2) the absorption is greatest where the skin is most delicate (axilla, loins, inner surface of the extremities). Absorption is aided by rubbing.

Watery solutions as a rule are not absorbed from the skin but when the solutions are brought in intimate contact, certain salts may be absorbed. Skin is capable of absorbing sulphuretted hydrogen and other gases. Application of watery solutions to the abraded skin or open wounds is, however, quite a digerent matter. Absorption under such conditions, takes place readily and the rate of absorption is practically similar to that obtained by subcutaneous injections. Chronic and callous ulcerations with defective circulation are, however, poor absorbing surfaces.

(2) On Mucous Membranes

Absorption from mucous membranes.

Drugs can be applied locally to surfaces other than skin, *e.g.*, mucous membranes. The conjunctiva absorbs readily, so that apart from local action, a systemic effect of drugs may, sometimes, be produced, *e.g.*, atropine. A few milligrams of apomorphine in the conjunctival sac of a dog produce vomiting. The vagina absorbs freely and clinical poisoning may be produced by giving the drug in the form of douches. The uterine cavity also provides a very good absorbent surface. The urethra absorbs readily and frequent poisoning has occurred from application of local anæsthetics. The urinary bladder absorbs poorly; drugs like strychnine, apomorphine, morphine, etc., are quite harmless when given by this way. The ureter and renal pelvis absorb fairly well.

The lungs also absorb fluids and dissolve substances rapidly if these are introduced through the trachea. A continuous stream of warm water given into the trachea of a horse, at the rate of 6 litres per hour was absorbed; no trace of water being found, though the animal died of direct injury to the lung tissue. Rabbits can receive 30 to 40 c cm of fluid into the trachea. If the fluid is injected more rapidly than it is absorbed it fills up the alveoli and produces asphyxia. This route of administration is, however, not used in therapeutics on account of the danger of asphyxia.

Sprays.

Sprays are finely atomised solutions and are inhaled for their local action on the mucous membrane of the nose, pharynx and larynx. They must not be too irritant. To reach the lower air passages they should be deeply inhaled with the nostrils closed, the mouth widely opened and the tongue slightly protruded. In the treatment of asthma 1 in 100 adrenalin spray introduced by an atomiseris used with benefit. Small quantities 2 or 3 c cm of oily solutions (*e.g.*, menthol) may be injected directly into trachea through the glottis. They are then distributed by respiration. Powders are also frequently blown on to a surface, *e.g.*, boric acid into the ear or relatively inaccessible parts and this is called insufflation.

Inhalations.

Inhalations are used for gaseous medicines such as anæsthetics, oxygen, etc. The effect depends on the concentration of the gas and the time during which it is administered. The action is most rapid as they reach the blood stream quickly. The capillary surface of the lungs, which is only separated from the alveolar air by a single layer of flattened epithelial cells, is wonderfully adapted for absorption of volatile substances into the blood. The chief advantage of this route of administration is the possibility of controlling the amount of drug at any desired time. The effect also disappears quickly after the drug is discontinued.

A recent advance in this direction has been the use of trichlorethylene inhalations for trigeminal neuralgia. The patient in attack, after breaking a 1 ccm capsule keeps on inhaling the drug till the sweet and distinctive odour has disappeared. Inhalations may be repeated upto four times in 24 hours. Relief is claimed to be prompt and lasting for some time. If the drug is inhaled every day, using 2 to 4 ccm a day, for 4 to 6 weeks, some cases are claimed to be entirely cured. Another recent use of inhalation therapy concerns benzedrine (benzyl-methyl-carbinamine). Nasal inhalations of the latter drug can relieve conditions of the nose where a vasoconstrictor effect is desired (*e.g.*, hay fever). Its action is similar to that of ephedrine or adrenaline.

Local.

Inhalations are used for local action also. These can be effectively employed by evaporating the substance with steam, either by placing it on boiling water and inhaling it through a funnel, or by the steam atomizer. The steam gets condensed on the surface of the

mucous membrane and the drug is deposited at that area. Even with deep inhalations the vapour does not go beyond the larger bronchi. The respiratory movements, however, tend to distribute the material widely through the lungs. In many affections of the nasal and upper respiratory passages this method is very commonly used.

(3) Ionic Medication

Definition.

By this is meant the administration of drugs, for either local or general effects, through the skin and by the use of electric current. Proper manouvring of cathode and anode is all that is necessary to lead the dissociated ions of electrolytic solutions, into the tissues. Superficial or even deep ionisation may be used when superficial effects are desired but for systemic effects deep ionisation is required.

Mechanism.

Negative charges (acid radicals) are attracted by the anode and positive ones (basic radicals) by the cathode. For instance, in the case of potassium iodide (potassium has a positive charge whereas iodine has a negative one) when the drug is applied to the surface of the body by the anode, the cathode being placed at any other point, the potassium (+) will collect at the cathode (—) after penetrating the tissues, whereas iodine (—) after dissociation, will collect at the anode (+). Similarly iodine can be led into the tissues by applying the drug by the cathode. The quantity of ions which can be taken into the tissues is dependant upon the strength of the current used.

Therapeutic field.

When salts of organic bases are being used for ionic penetration, they should be applied at the anode as in their case the basic radical is the one which is generally needed to be driven in (*e.g.*, aconite, cocaine, morphine, etc.). In the case of inorganic compounds (*e.g.*, bromides, iodides and salicylates) acid radical is usually the one required and thus they have to be applied at the cathode. The therapeutic field for ionic medication, though limited, does exist. It is superior to topical applications and may be used in selected cases of arthritis or an indolent ulcer. For ionic medication in human beings, a current of 2 milliamperes per square centimeter of active electrode, for 15 to 20 minutes, should not be exceeded.

3. Oral and Rectal Administration

Oral administration is the most ancient method and is still the method of choice with most drugs. Drugs whose general action is desired after absorption are generally given by this way, but sometimes drugs may be given for their local effect by this route also (*e.g.*, bismuth and emetics for their action on the stomach, vegetable purgatives on the intestines).

Some drugs give rise to disturbances of digestion when given by the mouth. This can be avoided by giving them in the form of pills coated with keratin, in capsules or cachets so that they will not get dissolved in the stomach, or they may be given when the stomach is full so that they are diluted. Absorption mainly takes place from the small intestines.

Absorption per rectum.

Rectal administration may be used to avoid the action of a drug on the stomach and the small intestine. The rectum, on account of its profuse vascularity and venous plexuses, is a very good absorbing surface for many soluble substances so that effects are often more prompt and more marked than with the oral method. Absorbed substances moreover do not all pass through the liver where they are liable to be destroyed. The drug is given in the form of an enema or suppository. Enemata when given for absorption should be as small as possible (2 ounces) and not irritant. The rectum should be washed out with warm normal saline before they are given. About twice the dose given by the mouth is necessary to produce the usual effects.

The rectal administration of drugs has been practised since the time of Galen and although its utility is limited, it still occupies a very large place in therapeutics. As many drugs are fairly well absorbed from the rectal mucous membrane, there is a growing idea that this route of administration may be resorted to in supplying nutriment to the system whenever occasion demands it.

Rectal feeding.

The use of nutrient enemata or suppositories containing food meterials is based on the assumption that absorption takes place from the rectum and the large bowel. The absorption of foodstuffs from the rectum is, however, very limited. Physiologically, the rectal mucous membrane is unsuited for the purpose of digestion and absorption of nutrient material. The main digestion of food, as is well known, takes place in the small intestines. The pancreatic juice furnishes powerful ferments which digest starch (amylase), fats (lipase) and proteins (trypsin). The bile renders important assistance in the digestion of fats. The secretion of the mucous membrane of the intestines (succus

entericus) also possesses an important digestive function through its ferment 'erepsin.' After the digestion is completed, absorption takes place through the highly specialised epithelium of the villi of the intestinal mucosa. Practically the whole process of digestion and absorption is completed in the small intestines before the material is transferred through the iliocæcal valve into the large intestines. In the large intestines absorption of water chiefly takes place. 'Erepsin' is the only ferment present which can break down proteoses and peptones only into the simpler amino-acids. The villi are widely different and are unsuited to the absorption of nutrient material.

Protein absorption.

The bacterial contents of the large intestines may play a part in digesting certain materials which are resistant to the action of the digestive juices in the small intestines, *e.g.*, cellulose, but this digestion is very insignificant in human beings.

Some observers have stated as a result of comparative study of the nitrogenous metabolism in patients on rectal feeding, that quite large amounts of protein are absorbed. Recent observations have, however, demonstrated that this statement was based on fallacious experimentation and cannot be taken at its face value. Boyd has shown that nitrogenous equilibrium could not be obtained during rectal feeding even in those who were accustomed to a diet poor in nitrogen. Laidlaw and Ryffel estimated the nitrogenous output in a case of rectal feeding during coma, and found that it was approximately equal to that obtained in the later stages of fasting, indicating that protein absorption was not taking place. Langdon Brown has carried out a number of experiments by giving nutrient enemata consisting of milk, plasmon, dextrose, sodium bicarbonate and liquor pancreaticus. He estimated the nitrogenous output in the urine, as this is a more accurate criterion of the absorption of nitrogenous material from the bowel than the loss of nitrogen from the rectal washings, which method was employed by previous workers. It was found that the nitrogenous metabolism was hardly affected.

Carbohydrate absorption.

Normally, carbohydrates are absorbed by the bowel as dextrose, and of all the foodstuffs, this appears to be very well utilised in rectal administration. That dextrose is definitely absorbed from the bowel is proved by the fact that the respiratory quotient was raised and that ketosis could be definitely abolished. A 6 per cent solution of dextrose in 15 ounces of tap water is used six hourly, with daily irrigation of the bowel four hours after the last dose. Different observers have claimed that nearly 67 to 100 per cent. of the carbohydrates are absorbed.

Fat absorption.

As is well known, fats are never absorbed *in toto* but are first broken up into glycerin and fatty acids through the agency of an enzyme 'lipase.' This ferment is not present in the large intestines but is supplied by the pancreatic juice. Bacterial decomposition may, under exceptional circumstances, break up the fats but this is not of much significance. Even if the pancreatic ferment is provided by the addition of liquor pancreaticus, absorption of fats is not satisfactory. The fat in the yolk of the egg is considered to be better absorbed than other forms of fat and has been widely employed. Eggs are, however, not absorbed and most of them are thrown out and add to the nursing difficulties without any advantage to the patient. Though the earlier reports indicate that partial absorption of fats takes place, recent observations definitely negative the suggestion. Practically no fat is absorbed from nutrient enemata by the large bowel.

Salts and water.

It is agreed by all physiologists that salts and water are freely absorbed from the large intestines and the advantages claimed for rectal feeding are probably due to these ingredients. It is well known that the body can stand deprivation of food for a considerable time if these articles are supplied. The administration of 10 oz. enemata of plain water at a temperature of 100°F every four or six hours has been advocated.

It will, therefore, be seen that the principle of rectal feeding is physiologically not very sound. It is only a poor substitute for oral feeding, as absorption from the small intestine is completely cut off. It has been claimed that the larger enemata will get through the ileocæcal valve, and be absorbed in the small intestine. It was noted in a case of duodenal fistula that some quantity of a soap and water enema reappeared through the opening. This regurgitation of rectal enemata is such an exceptional circumstance that no reliance can be placed on it. We have, therefore, to depend on the capacity of the rectal mucous membrane to absorb the foodstuffs. As the large intestine is the principal place for the absorption of water, it is essential to supply the nourishment desired, in fluid form. But even in this form, its utility is limited. If any protein is absorbed from the ordinary nutrient enemata, the amount is so small as to make it hardly worth while to subject patients to so much discomfort for so small an advantage.

It appears possible that better results might be obtained if the proteins are completely broken down into amino-acids, the form in which absorption normally occurs.

By allowing pancreatic extract to act on milk for twenty-four hours and using the digested material as an enema, decidedly better absorption takes place.

Disadvantages.

It is true that water, salts and dextrose can be absorbed from the large bowel in sufficient quantities to tide the patient over periods of difficulty and may avoid ketosis when for some reason or other, the patient cannot take anything by the mouth. There are, however, certain disadvantages which should be borne in mind. Apart from the inconvenience and difficulty caused in keeping the patient in a clean condition, there are certain symptoms which require attention. Excessive thirst, persistent vomiting, reflex secretion of gastric juice, parotitis due to ascending infection of the salivary ducts, etc., are some of the unpleasant complications. These can be avoided to a large extent if proper nursing facilities are available.

4. Administration of Drugs by Injection

This method is of comparatively recent origin, but its use is being rapidly extended.

Advantages.

Irritant drugs should not be given by injection into the tissues, as this may cause great pain and swelling and sometimes suppuration, even when all precautions regarding sterilisation are taken, *e.g.,* turpentine, and digitalis glucoside. The advantages of the injection method are:—

(1) Certainty of action, as all the drug gets into the tissues and therefore the dose is definite.

(2) Rapidity of action, as the drug quickly reaches the circulation.

(3) When administration by the mouth is not possible, as when the patient cannot swallow (unconsciousness, drunkenness, etc.) or when the alimentary tract cannot tolerate or absorb drugs (uncontrollable vomiting and diarrhœa).

Disadvantages.

The disadvantages are:—(1) Abscess may form at the site of injection if precautions are not taken, (2) the drug may be injected by mistake into a vein with disastrous results, (3) the drug may be injected into a nerve giving rise to excruciating pain or paralysis (*e.g.,* quinine). All these can be avoided if sufficient care is taken.

Syringe.

Only the best instruments make possible a good technique, and on the technique depends a good deal of the success of the therapy. It is not essential to possess a 'record syringe.' An all-glass syringe may serve the purpose even better provided it is made of alkali-free, hard (resistance) glass. The graduation and capacity of the syringe must be accurate. Tips of all-glass syringes break easily if not properly ground, or if the size and taper is not such as to fit securely and tightly into a needle. The size of the tip should not be too long (*i.e.,* not more than ½ inch. for 2 and 5 ccm syringe and 17/32 inchs for 10 ccm syringes). Proper and secure joint can also be afforded by a properly designed "lock" to fit the commonly used standard needles. Proper fitting of the tip of the syringe with the needle prevents tip breakage, leakage of solution and 'coming off' of the needle. Some simple tests may help in the choice of a good syringe. Draw up slowly some water and see that no air intervenes between the piston and the fluid. Free working of the piston should also be tested. The opening of the tip of the syringe should be of the same inside diameter as that of the largest needle used with each size syringe. The fitting between the barrel and the plunger should obviously be neither too loose nor too tight.

Accuracy of graduation of the syringe can easily be checked up by a standard measure. A fine black line at the base of the plunger facilitates reading and regulation of dose. For testing the glass for free doses from alkalies the Standard Phenolphthalein Test of the United States Government may be employed.

(1) Sterility of Syringes

Sterilization of syringe.

There is much doubt and misconception about the efficacy of different methods of sterilizing svringes. The Committee of the Medical Research council in its memorandum entitled "The Sterilization, use and care of syringes" has given a comprehensive survey of measures to ensure sterility. There are also sections on the choice of syringes and needles, the care and sharpening of needles; the methods of testing syringes for leakage; the organization of a clinic for mass inoculation and the precautions necessary in employing syringes for certain special purposes such as tuberculin tests; the withdrawal of blood for anti-toxin estimations, and mass intra-venous injections.

The first injunction is that *syringes used for aspirating septic material, should never be used for injection of a sterile medicament.* The neglect of this elementary precaution

Autoclaving.

may cause terrible disasters. There should be sufficient number of syringes and separate ones for different jobs. It is urged that there is no known method which can be absolutely depended on to sterilize a syringe except autoclaving or exposing to a temperature of 160°C in a dry oven. This seems to sound the death knell of the record syringe, since cement at the glass-metal junction is liable to melt at these temperatures. In fact all-glass syringes of a two piece type are advocated. The all-glass syringe is easily cleaned and has no obscure crevices in which previous contents may lurk. The best thing is to have plenty of such syringes, to encase them in glass tubes and to sterilize them by dry heat. It is advocated that in a large hospital a single department should have the care of all the syringes, cleaning, sterilizing and re-issuing them for use.

Boiling.

Boiling is given as a second best alternative; it will not kill sporogenous bacteria unless sodium carbonate is added to the water; this however is not recommended as the resulting alkalinity of the syringe may affect the solution to be injected. The risk of infection by sporogenous bacteria, though remote, exists. For this reason the dry method is preferred to a wet one. The hot oil method was tested and found to be ineffective.

Use of alcohol.

Chemical methods of sterilizing a syringe, such as with hypochlorites, phenols, chloro-phenols, etc., are ineffective and may damage the material to be injected. Besides this they do not penetrate the crevices in which bacteria may lodge. The only chemical disinfectant receiving even a qualified recommendation is 70 to 75 per cent V/V alcohol and this should be only confined to syringes employed for and by a single patient for the injection of insulin and adrenaline. Alcohol acts best in a concentration of 75 per cent by volume in water, and scarcely at all when undiluted. It acts only on vegetative bacteria and has little power of penetration. It is a better disinfectant than most for skin, but unreliable when absolute sterility is necessary as for instruments. It should also be remembered that when the skin is moist, as in hot climates, undiluted alcohol is effective and the concentration used should not be less than 80 per cent owing to dilution with sweat. Ether has been tried as a skin disinfectant and has been found to be ineffective.

Injection material.

Regarding solution for injection, care should be taken not to include any solid particles from ampoules, or tiny masses of undissolved drugs, which when present should be filtered out before injection.

A cold compress at the site of injection or application of an elastic bandage above the injected part will be found useful when it is desired to reduce the rate of absorption.

When a syringe becomes jammed any of the following procedures may help. Boil in 25 per cent aqueous solution of glycerine and remove the plunger while still hot. Put nitric acid or ether around the head of the syringe tip and allow it to permeate in between barrel and plunger before they are separated. If it is a record syringe put it in the refrigerator for sufficient length of time, and later draw out the plunger. Stains on the syringe can be removed by nitric acid.

Needles.

A good needle should have a clean cutting edge, flexibility, and strength. The needle point should have no irregularities. These can easily be detected by a hand lens, or by drawing the needle back across a piece of gauze or the finger nail. Needle points have varying types of bevels: the long bevel for aspiration, subcutaneous and intra-muscular use and the short bevel for going into the vein. Extra short bevel is recommended for intradermal and spinal use. Regarding the size of the needle, the depth of the tissue being aimed at, safety rate of flow and lastly the question of pain to the patient, should be borne in mind. Extended use weakens the needle and increases chances of breakage. Before sterilizing, every needle should have its flexibility tested; this can be carried out by bending the shaft of the needle forwards and backwards. A steel needle three inches long should be able to make an arc of about 45° and spring back to normal; smaller needles would obviously make smaller arcs. For keeping needles free of rust etc., these should always, after use, be syringed out with water, alcohol and ether, so that they remain dry.

(2) Subcutaneous or Hypodermic Route

Subcutaneous route.

In this method, the drug is given into the subcutaneous tissues by means of a hollow needle. All preparations meant for subcutaneous injection should be in liquid form, solutions or colloidal suspensions and should be capable of complete absorption, for otherwise they will set up irritation. The quantity injected should not be too large (under 5 ccm). Large quantities of saline are sometimes injected into the loose tissue about the

breast, axilla, abdomen or into the back below the scapulæ, the liquid being allowed to run in slowly. Care should be taken that the fluid is neutral in reaction and is isotonic or nearly so with blood, for otherwise it will not be absorbed and pressure on vessels may cause gangrene or abscess.

(3) Intradermal Route

Intradermal route.

Intradermal injections, apart from a few therapeutic or prophylactic uses (*e.g.*, small pox vaccination, Besredka's antivirus, leprosy, and certain foreign proteins, etc.) are mainly employed for diagnostic purposes (*e.g.*, Schick, Dick, and various allergic tests,). The injections are given on the flexor aspect of the forearm generally and when the needle is in the proper position the opening in the bevel should be visible through the epidermis. The quantity of the fluid injected is specified in the tests but does not exceed 0.2 ccm. Details of some of the important tests are given in the dictionary.

(4) Intramuscular Route

Intramuscular route.

The injected substance ruptures the muscle bundles and spreads along the nearest fascial planes thus greatly increasing the absorbing surface. The absorption, therefore, is more rapid than with subcutaneous administration and in view of the fact that sensory nerve-endings are fewer than in the skin, there is less pain. The tendency to abscess formation is also much less. Intramuscular injections are particularly useful when relatively insoluble substances suspended in oil have to be administered. These establish a depot for gradual absorption and continued action, *e.g.*, mercury salicylate in the treatment of syphilis. Suspension of metallic salts in oil are best injected intramuscularly as they are hardly absorbed from the subcutaneous tissues.

Technique.

Intramuscular injections are made by thrusting the needle through the skin, deep into the substance of the selected muscle. The buttocks are most commonly selected for these injections and the most favourable site is the upper and outer quadrant. If the injected material is deposited near a nerve root, nerve plexus or nerve trunk, obstinate neuritis may result and if deposited on the fascia of the region, superficial nodule formation, abscesses, or deep firm indurations may develop. The tissues at the selected site should be flattened and fixed with the palm of one hand and the needle inserted at the selected point. The plunger should be withdrawn slightly to make sure that the point of the needle has not entered a blood vessel. The injection is then given slowly. With proper technique, the flow of the injected material is always free and unobstructed.

Leakage prevention.

Provided the needle points and syringes are mechanically perfect and the buttocks have not been rendered fibrous by repeated injections, there should be no leakage along the needle track. Excepting where the buttocks have been rendered fibrous the leakage may be prevented; by securing complete relaxation of the musculature, by the use of a small-gauge needle, by completely emptying the needle before rapid withdrawal, by pushing upward of the tissues pulled down by the left hand, and lastly by gentle massage at the point of puncture, with a pledget of cotton wool saturated with alcohol. When repeated courses of injections have rendered the buttocks fibrous, leakage cannot be prevented and ultimately intramuscular injections must be discontinued in this region.

Complications.

It must be remembered that due to faulty technique, worn out needle, or want of co-operation of the patient, a needle may break inside the muscle. This is usually due to ageing of the needle which is thus rendered brittle. The usual break is at the junction of the shaft and the hub. This complication is mostly prevented by the use of best makes of needles or the use of safety needles, with bead on the shaft, and, in either case the needles should not be very old. As already stated persistent nodule formation, abscess formation and neuritis are other complications.

(5) Intravenous Route

Intravenous route.

During recent years, the practice of intraveous medication has come to the forefront. Twenty years ago, medical men would not willingly undertake the injection of a drug into a vein. Now-a-days, however, the popularity of the methods has increased and the modern physician performs this operation with alacrity and skill. Attempt is made to give almost every drug by this route on the assumption that intravenous medication produces a more powerful therapeutic effects than administration of drugs by other channels. This misconception has assumed such proportions that it is both timely and important to consider the real merits and limitations of this form of therapy.

Historical.

The idea of intravenous medication was first started in 1655 when Christopher Wren experimented on dogs and showed that drugs could be given into a vein without ill effects. A year later the first intravenous injection was given in man. The discovery of salvarsan in the beginning of this century and its safe intravenous use put intravenous

therapy on a sound and stable basis (1910). The demonstration of the relative harmlessness of intravenous salvarsan injection came as a great relief to the physician who could thus treat syphilis without giving the patient the excruciating pain attending on intramuscular injections.

Physiological considerations.

A knowledge of the inherent properties of the blood is essential in order to have a closer conception of this method of administration. A brief reference to the physiological considerations will not, therefore, be out of place here.

The blood is the chief medium through which any drug introduced by any channel will be distributed to different parts of the body. It is a special fluid, having the function of carrying nutrition and oxygen to all the tissues and eliminating the waste products that accumulate as a result of metabolism. It also takes certain chemical substances that are formed by physiological activity of an organ to other organs, thus regulating and co-ordinating the functions of different organs of the body. The total volume of blood has been estimated approximately to be 1/13 of the body weight. The erythrocytes form about 50 per cent of the total mass and consist of two parts, stroma and haemoglobin in a state of loose combination. This combination may be broken up by various means such as dilution of the blood with water, addition of ether, or bringing it into contact with haemolysins. Laking of the blood by dilution with water makes the consideration of the osmotic relations of the erythrocytes an important study in intravenous injection. If the osmotic pressure of the blood plasma is reduced by diluting with water, the corpuscles in order to maintain the balance, break up liberating their haemoglobin. If a strong solution of common salt is added to the blood so as to make the osmotic pressure of the plasma higher than that of the erythrocytes, water will pass from the corpuscles to the plasma to maintain the equilibrium and the corpuscles will be crenated. The osmotic pressure of both the plasma and the corpuscle will, therefore, remain unaffected between these two extremes and this point is reached by a solution containing 0.9 per cent of sodium chloride, for mammalian blood. The erythrocytes are impermeable to most neutral salts and, therefore, it is possible to make normal solutions with sodium sulphate or with cane sugar by making the solution isotonic with 0.9 per cent sodium chloride. Certain substances such as alcohol, ether, etc, however, can permeate through the corpuscles. The blood corpuscles resemble most animal and vegetable cells in permitting the passage of those substances that are soluble in fat and fat-like bodies, *e.g.,* lecithin and cholestrin.

Specific gravity.

The specific gravity of the blood in the male sex varies between 1056 and 1058 and in the female sex from 1054 to 1056. The question of specific gravity becpmes important when large quantities of saline are infused intravenously as in cases of cholera. If the specific gravity of the blood is high enough, as it is in this disease, very large quantities of saline can be injected without any untoward effects. When the specific gravity is within normal limits, administration of large quantities of saline is liable to produce œdema of lungs and death.

Reaction of blood.

The hydrogen-ion-concentration of the blood is another important consideration in intravenous therapy. The blood, as is well known, is alkaline to litmus. The pH of this fluid depends mainly upon the ratio between the concentration of carbon dioxide and bicarbonates in the blood, and these two are so balanced that the pH is just on the alkaline side of neutrality. This balance may be expressed as an equation: pH (hydrogen-ion-concentration) $= K. \frac{H_2CO_3}{NaHCO_3}$ where K is a constant. An addition of a trace of acid or alkali will disturb the balance by increasing or decreasing its numerator or denominator and make the pH move towards the acid or alkaline side of neutrality. The tissues and especially the higher centres in the medulla are extremely sensitive to the slightest changes in the hydrogen-ion-concentration of the blood, and any change in the relative proportions of H and OH ions which will take the pH of the blood to the acid side of neutrality will be met with rapid fatal results. Under ordinary conditions, therefore, intravenous injection of any acid solution would have been impossible. The blood, however, contains certain substances called 'buffers' which play an important part in maintaining the pH of the blood at a constant level. The mechanism by which the neutrality of the blood is maintained is described in detail in chapter on acidosis and alkalosis.

The balance of pH is so delicately maintained, that ordinary injection of drugs which are distinctly acid or alkaline produce no permanent change in the reaction of the blood. If, however, the rate of injection is rapid, the buffers present may not be able to keep the balance intact.

Blood volume.

The volume of the blood is another important subject for detailed study in connection with intravenous therapy. It is a well-known fact that the blood volume remains fairly constant despite influences which tend to alter it. After a severe hæmorrhage, the volume of the blood is made up by gradual imbibition of fluid from

the tissues. Again when large quantities of saline solutions are injected into the circulation, the total blood volume does not alter permanently. However, the effects produced immediately are dependent on the strength of the saline, its temperature and on the speed with which it is driven in. A hypertonic solution attracts fluid from the tissues, brain and cerebrospinal fluid, into the blood circulation but in case of hypotonic solutions the reverse happens and some water is drawn out from the blood into the tissues, brain and the subarachnoid space. If the temperature of the injected fluid is higher than the body temperature a direct action on the cardiac centre and the sinoauricular node is produced and the heart accelerates; with fluids colder than the body temperature the heart is slowed. Regarding the speed with which the fluid is injected, if it is rapid the venous pressure is raised and as ultimately more blood is injected into the arterial system, blood pressure temporarily rises. With slow injections no such change is produced. In animals such as cats and rabbits, about 100 to 200 ccm of normal saline infused slowly do not produce a rise in arterial pressure proportionate to the quantity injected. The fluid, therefore, must be accommodated either inside or outside the vascular system. There may be dilatation of the arterioles and capillaries to make room for the extravolume of fluid or the fluid may pass out of the circulation into the tissues as lymph. Both factors appear to operate simultaneously. Immediately after such a transfusion an increased fullness in the vessels of the mucous membrane and retina can be observed. The fluid portions of the blood, however, rapidly pass from the blood vessels to the tissues giving rise to œdema, if the quantity of the fluid transfused is very large. This œdema might set in, in such vital organs as the lungs and may produce serious respiratory distress or even death.

Air-embolism.

The question of the entry of air into the circulation may be briefly reviewed here. It is a matter of common observation in laboratories that small quantities of air introduced into the circulation do not have any deleterious effect on an animal. On the other hand, injection of large quantities of air is the quickest and surest method of killing an animal; 5 ccm of air suddenly introduced into the vein of a rabbit will produce fatal results within 2 or 3 minutes. If, however, the air is introduced slowly the animal does not die. If 5 to 10 ccm of air are injected abruptly into the vein of a cat, an immediate fall in blood pressure to zero is produced. The churning of blood and air in the heart produces froth, and sudden heart failure may occur due to very small quantities of blood entering the coronary arteries with consequent impairment of the nutrition of the heart. It must be realised, however, that in order to produce such result in man, the quantity of air introduced must be very large. Five to 10 ccm of air, even when introduced suddenly, will be rapidly absorbed by the blood and will produce no deleterious effects whatsoever.

Selection of drugs.

The selection of the salt to be given when several salts of an alkaloid are available should depend upon the solubility of the salt and its rate of diffusibility. Generally, the hydrochlorides are more soluble and more diffusible than the sulphates because of the smaller size of their molecules. Another point in favour of hydrochlorides is that HCl combines with the calcium of the blood, forming calcium chloride which is soluble. The calcium content of the blood will, therefore, not be affected. If a sulphate is given, the insoluble calcium sulphate which is produced will be precipitated and will deprive the heart of its proper calcium requirements. The chemical changes, such as those occurring in the blood, however, are so insignificant with ordinary injections that their effects are negligible in view of the large volume of the blood in circulation.

Some salts such as the pentavalent compounds of antimony produce a precipitate in the blood. This precipitate may produce respiratory distress but the symptoms pass off rapidly and generally produce no untoward effects. Substances such as salvarsan are not soluble in neutral watery solutions and therefore the blood cannot hold them in complete solution. They circulate in the blood in a semi-colloidal state.

Indications.

The following are some of the chief indications for the administration of drugs by the intravenous route.

1. As an emergency measure when rapidity of action is desirable. A few examples may be given to illustrate the point:—(*a*) For combating acidosis in the body, *e.g.*, in diabetic coma, the intravenous infusion of saline, sodium bicarbonate, insulin, etc., may save the patient's life. (*b*) In severe forms of tetany, intravenous injections of calcium may prevent death. (*c*) In auricular fibrillation with impending death, an intravenous injection of strophanthin may act like a charm. (*d*) In malignant malaria, intravenous administration of quinine or atebrin produces rapid curative effects. (*e*) Severe cases of diphtheria generally demand the intravenous use of antitoxin, and often respond favourably.

2. When greater intensity of action is desired than can be secured by other methods of administration. Arsenicals are given in syphilis by this route to produce an effective concentration in the blood. Antitetanic, antimeningococcic and antistreptococcic sera (especially for scarlet fever) are sometimes given intravenously to produce stronger action than can be obtained when given subcutaneously. Non-specific proteins, when used to induce protein shock, are best given into the vein, *e.g.*, typhoid vaccine in chronic arthritis.

3. To secure direct action on the infecting organisms within the blood stream, *e.g.*, quinine in malignant malaria.

4. To avoid irritation and destruction of tissue likely to be produced by drugs, such as arsenicals and antimonials, when given by other routes.

5. When the volume of the fluid is large it is better to give it intravenously than intramuscularly or subcutaneously, *e.g.*, injections of saline in cholera. Though large quantities of saline can be given under the breast and in the loose areolar tissues of the axilla, the procedure is very painful and should be avoided as far as possible. The various advantages of intravenous therapy over other modes of administration are:—

Advantages.

1. Precision of dosage. This can only be attained by injecting a drug straight into the blood stream. There is no certainty what amount of a drug administered orally is going to be absorbed finally into the circulation.

2. Absence of gastro-intestinal irritation: Some arsenicals and antimonials cannot be given by the mouth.

3. Stability of the drug, *e.g.*, adrenaline and many hormones are rendered inert when given by the oral route.

4. Prompt action.

5. Avoiding irritation or destruction of tissue when given by subcutaneous or intramuscular method.

Disadvantages.

Intravenous medication should not be resorted to if a drug can be effectively given by any of the other routes, as there are many risks attached to this form of medication. There is a growing tendency on the part of medical practitioners to resort to intravenous injections when the utility of this method is doubtful. This is to be strongly deprecated. The injection of foreign substances into the blood stream should always be considered a serious undertaking and the advantages and disadvantages should be carefully weighed before it is actually practised. The risks of intravenous injection frequently outweigh its probable benefits in (a) greatly debilitated patients. (b) the aged, (c) patients with hypertension and arteriosclerosis, (*d*) patients suspected of being subject to anaphylactic reactions, *e.g.*, asthma, urticaria, sensitiveness to drugs. Furthermore, the injection of substances likely to disturb or produce a subversion of the delicate physiological balance of the blood is contra-indicated. Substances markedly acid or alkaline in reaction, imperfectly purified substances, substances which do not dissolve in water, hæmolytic agents, emulsions of fats and oils set up untoward effects which may be serious.

Technique.

The best veins to choose are those at the bend of the elbow, but if the patient is well covered these veins may be buried in fat and it may be easier to puncture one of the veins on the back of the hand. The latter are very moveable and it is often very difficult to puncture the vein, even when the needle is through the skin, as it recedes in front of the advancing needle and then suddenly slips away from it. There is a large vein at the back of the wrist running over the outer side of the head of the radius between the tendons of the extensor carpi radialis longus and of the extensor policis brevis which is extremely useful, especially in children, in whom it is often as big as the little finger. The vein is not usually blue but can very easily be felt, and if congestion is caused it will stand out very prominently. The disadvantages in using this vein are that the skin over it is usually tough and there is a nerve just under it which may be accidentally injured.

The veins on the innerside of the ankle or veins of the neck are some of the other alternatives. Varicose veins should not be used. The skin over the vein should be sterilised with alcohol. If iodine is used it must be washed off with alcohol, otherwise it will increase the difficulty of seeing the vein. Dashing the skin with xylol will increase the visibility of the veins but this is seldom necessary. Engorgement is caused by putting a rubber ligature round the arm above the point at which the vein is to be punctured. This ligature must be tight enough to stop the arterial flow to the limb. Further conges-

tion may be caused by gentle upward massage or by rapid extensor and flexor movements of the limb by the patient. In very nervous subjects local anaesthesia by ethylchloride spray or by injection of one drop of 2 per cent novocaine may be given if feasible before venipuncture is performed. The patient should be either lying down or sitting at a table with the elbow on a small pillow. The syringe should be held in the right hand at an angle of about fifteen degrees with the skin surface and entered upwards and along the long axis of the vein. The point may enter the vein immediately or the vein may slip to one side; in the latter case the point of the needle must be made to follow the vein, pressing into the side of the vein until it is pierced. The level of the needle should point upward and the mark on the syringe should be facing the operator. Directly the vein is punctured, blood will enter into the barrel of the syringe, which has been previously loaded with the dose that is to be injected. The congesting band is now released and the solution slowly injected. The injections should in all cases be given as slowly as possible; not less than two full minutes should be taken over the injection of a maximum dose. When once the point of the needle is in the lumen of the vein, every effort must be made to prevent its either slipping out again or being pushed through the opposite wall of the vein. During the loosening of the constriction and the pressing home of the plunger the operating hand must be steadied by being rested on the arm of the patient. If at any time during the operation there is any doubt as to whether the point is within the lumen of the vein, the operator can satisfy himself by slightly withdrawing the plunger again. If the needle is still in position blood will again flow into the syringe. Great care must be taken that no air escapes into the vein from the syringe.

In children, who are very likely to wriggle during the operation, a good method is to hold the syringe in the right hand and with the left hand grip the arm so that the back of the elbow lies in the hollow of the hand and the first two fingers and the thumb can be approximated in front of the elbow; as the needle is passed into the vein the barrel of the syringe is gripped between the fingers and thumb of the left hand so that the syringe and the arm cannot possibly move independently. It will not be found necessary to grip so tightly that the venous flow is stopped.

The drugs used must be of established purity determined by chemical or biological methods and should be freely soluble in ordinary solvents. Substances which are imperfectly soluble should on no account be injected into the vein unless they are in a colloidal state.

The solution.

(*a*) The water used for the solution of the agent to be injected must always be double distilled, sterile and free from pyrogens.

(*b*) Sterility of the solution just before injection is essential. This may be attained by autoclaving the solution if there is no chance of the drug breaking up by heat.

(*c*) The possibility of decomposition of the solution should be borne in mind. Heat converts a part of $NaHCO_3$ into a much more caustic and alkaline carbonate. Such substances as strophanthin are decomposed when their solutions are put up in soft glass ampoules, owing to the liberation of alkali from the glass. The physician should see if hard glass has been used for such ampoules before giving the injection.

(*d*) The reaction of the solution is important. All solutions for intravenous use should conform as closely as possible to the reaction of the normal blood which is slightly on the alkaline side of neutrality (pH 7.4).

(*e*) Solutions should always be as nearly isotonic with the blood as possible whenever the volume of fluid to be injected is large. Exception to this are conditions such as cholera and high intracranial pressure, when hypertonic solutions are necessary.

(*f*) The rate of injection of the solution should be slow in order that the circulation is not overwhelmed with large volumes of the fluid which it cannot cope with rapidly. Injection at a slow rate is specially important in connection with potent drugs like arsenicals, and antimonials.

(*g*) The temperature of the solution should be approximately that of the body, otherwise unpleasant reactions might result.

Risks.

There are various risks attendant on intravenous therapy and in order to avoid them those using the method should thoroughly familiarize themselves with the details of the technique. When it is realised how well the equilibrium in the blood is maintained, the grave consequences of its disturbance, the relative ease with which it may be upset and the serious accidents which may follow, will be obvious.

Reactions.

Anaphylactoid phenomena evidenced by rigors, chill, vomiting, sweating and pyrexia may follow immediately after intravenous injection, especially if large volumes of substances such as saline or glucose are given. Such reactions are very disturbing and their real nature is not clear. It has been

shown that distilled water frequently contains a pyrogenous body which is the product of its contamination with living organisms. This pyrogen is produced by the growth of the organisms and is not present in freshly distilled water. The water used should therefore be freshly distilled, unless it is properly preserved. The other view is that the anaphylactic reactions are the result of physico-chemical disturbances in the plasma set up by rapid changes in the blood. The temperature of the fluid at the time of its entrance into the vein is also important. It should be realised that the temperature of the solution in the funnel or glass barrel used in the gravity method is not the same as that at the tip of the needle, for the solution cools down during its passage through the rubber tubing.

Hydraemic plethora.

No agent should be used for intravenous injection which has got any deleterious effect on the circulation and is therefore likely to produce dangerous reactions. A dangerous condition from the intravenous use of sodium chloride in large quantities has been described. If the total quantity injected is very large—10 gm or more—a condition of 'hydræmic plethora' is likely to be induced by dilution and increase in the total volume of the blood. It has also been shown that the specific gravity falls from 1064 to 1054 after an injection; the kidneys and lymph channels promptly excrete the excess of fluid and may overshoot the mark, so that eventually the specific gravity may rise to 1067; the total blood is less in bulk and even more concentrated than it was before. This does not occur if the supply of fluid is kept up by administering saline per rectum.

If the kidneys are not capable of excreting water and salt quickly enough, there will be water-logging and some degree of dropsy may occur. This may take the form of fatal œdema of the lungs. This danger has frequently been met with following saline transfusion, especially in patients with nephritis.

Thrombosis and embolism.

Thrombosis and embolism have frequently been mentioned as the most dangerous outcome of intravenous therapy, and numerous instances of death following thrombosis and embolism of the pulmonary capillaries have been recorded. It is possible that sudden death might follow intravascular clotting and embolus might stop the heart by blocking the coronary arteries. But serious results attributable to thrombosis or embolism are seldom met with in practice and one or two bubbles of air injected with the solution in the syringe do not produce 'air embolism.' Nevertheless there is a chance of thrombosis starting at the site of injection, if the agent injected is sufficiently irritant to cause damage to the endothelial lining of the vein. To prevent this, an additional quantity of saline should be injected to wash down the irritant drug. This is the usual practice with drugs like salvarsan.

Leaking punctures.

There are other associated dangers not directly due to the injection but the results of faulty technique, occuring especially with those who are not experienced in the art of intravenous injection. The injection of drugs such as arsenic, antimony, calcium and iodine, into the perivascular tissues is fraught with serious results in the shape of acute inflammation and abscess formation requiring surgical intervention. Fibrosis (local) leading to limitation of movement of the elbow joint is not an unusual complication. Tetanus is also known to have occurred as a result of injection with imperfectly sterilised syringe and needle or improper attention to sterilisation of skin. All these dangers can be prevented with proper care and attention on the part of medical practitioners.

(6) Other Routes

Subarachnoidal.

In addition to the preceding main parenteral routes of drug administration there are a number of others which are resorted to occasionally. A drug may require to be introduced directly into the subarachnoidal space and a lumber or a cistern puncture may have to be performed. Spinal anaesthetics, antimeningococcal serum, tetanus anti-toxin, penicillin, etc are the usual remedies which are given by this route.

Intracardiac.

In some extreme situations, it may be advisable to inject such drugs as epinephrine direct into the heart. An intracardiac injection is usually given in the fourth left intercostal space, just inside the clinical apex beat, using a needle about four inches long. Before the injection, the position of the needle in the ventricle should be ascertained by aspirating some blood.

Intra-peritoneal.

Intraperitoneal injections are on rare occasions resorted to when other routes of administration are not available. The abdominal wall is entered in the middle line 1-2 inches below the umbilicus. Before an intra-peritoneal injection is attempted the urinary bladder must be emptied and the skin prepared in the usual manner. A fold of skin is picked up at the selected spot and the needle is inserted into its lower part from below upwards. In thin persons the passage into the peritoneal cavity can be felt. After the needle is in the correct position it may with advantage be fixed to the abdominal wall by adhesive plaster.

CHAPTER IV

CHEMOTHERAPY

HISTORICAL AND GENERAL DEFINITION AND SCOPE OF CHEMOTHERAPY—THEORIES OF PARASITOTROPISM AND ORGANOTROPISM: SIDE-CHAIN THEORY, CHEMICAL THEORY—FACTORS INFLUENCING THE ACTION OF DRUGS—DRUG RESISTANCE: GENERAL FEATURES, THE NATURE AND MECHANISM OF DRUG RESISTANCE, DRUG RESISTANCE IN TRYPANOSOMIASES, LEISHMANIASES, SPIROCHAETOSIS, AMOEBIC DYSENTERY, AND MALARIA—THE RETICULO-ENDOTHELIAL SYSTEM: INTRODUCTION AND GENERAL, PHAGOCYTOSIS OF PARASITIC ORGANISMS, RELATION TO CHEMOTHERAPY, CONCLUSION.

1. Historical and General

The search for a specific remedy to cure a specific disease or diseases is as old as the art of medicine. From very ancient times physicians and healers have worked to attain this ideal, and various remedies have been suggested and tried in the treatment of various diseases. In former days chance was the only foundation of their investigation, and empiricism dominated the therapeutic investigation in the treatment of disease. In fact, empiricism still dominates the scientific search for specific remedies and it has not been altogether unsuccessful. There are the examples of cinchona for malaria, salicylates for rheumatic conditions, ipecacuanha for amœbic dysentery, all of which were used empirically centuries ago. The number of specific remedies is unfortunately very small. The modern scientific line of investigation of specifics for diseases is known as 'chemotherapy.'

General Consideration.

Before entering into any details it will be convenient to see what the meaning of the word chemotherapy in the modern sense of the term implies. It has been observed before that the use of certain drugs in specific diseases is as old as the art of medicine. The work of scientists such as Pasteur, Koch, Lister and others advanced our knowledge of diseases to a considerable extent and the discovery of microbes as a causative agent for infectious diseases, with the observations about the action of chemicals on these microbes, indicated that the long-maintained theory of finding specific drugs for specific diseases was about to materialize. Unfortunately though certain chemicals such as phenol, and mercuric chloride, exerted a very powerful action on bacteria, their action on the body cells was equally marked and the chemicals highly toxic to bacteria were also toxic to the body cells. Their employment in practical therapeutics is, therefore, not possible.

The discovery of diphtheria antitoxin and its successful use as a curative and prophylactic measure against diphtheria, stimulated the idea of finding antibodies for all bacterial diseases through natural agencies, but it was soon realised that this theory does not hold good for the body does not develop immunity in the case of all diseases as it does in the case of diphtheria. It was then thought that rapid advance in synthetic chemistry might give that power into the hands of man which is absent in nature, that it might be possible to evolve certain synthetic products which would be very toxic to the parasites and least harmful to the body cells. This has come to be true.

Trypanocidal Action.

The father of chemotherapy was Ehrlich who in 1891 discovered that malarial parasite is stained by methylene blue. He then tried to find dyes which would kill micro-organisms without damage to the host. This work led to the discovery of the curative action of 'trypan red' in mice experimentally infected with *T. equinum*. After this followed trypan blue, afridol violet and several other sunthetic coal derivatives. Immediately following this work, Thomas (1905) found that atoxyl in combination with trypan red exerted a curative effect in laboratory animals artificially infected with different species of trypanosomes. This work eventually led to the synthesis of salvarsan and definitely established the modern science of chemotherapy.

Shortly after the discovery of atoxyl as a trypanocidal agent, experiments were conducted with spirochaetal infections. It was shown that certain compounds of arsenic, having no spirochaeticidal action *in vitro,* have marked curative properties in spirochætal infections *in vivo* especially those of relapsing fever in mice and spirillosis of chickens. The appearance of Bayer 205 for the treatment of sleeping sickness was a great step forward because this complex derivative of benzene and naphthalene contains no arsenic. Recently certain aromatic diamidines have been shown to be highly effective against certain protozoa. Although quinine retains an unassailable position in the treatment of malaria, many of its analogues have been ineffective. The synthetic anti-malarials plasmochin,

atebrin etc. were developed following the observation of Ehrlich with methylene blue. Paludrine has been recently synthesized and tested by workers of Imperial Chemical Industries.

The sulphonamide group.

The guiding hypothesis was always the idea of direct action of the chemo-therapeutic agent on the micro-organisms . For a long time there was no chemotherapy of bacterial infections although its possibility was recognised in prontosil, an azo-dye. During recent years a large number of sulphonamide compounds effective against bacterial disease have been prepared and are in use.

Antibiotics.

This was followed by preparation of antibiotic substances such as penicillin, streptomycin etc. by Fleming, Florey, waksman and others. These substances differ from sulphonamides in that they are bacteriostatic rather than bactericidal and their action is not interfered with in the presence of bacterial extracts, pus, fluids or p-aminobenzoic acid which hinders the action of the sulphonamide group. The outstanding problems among bacterial infections which still remain unsolved are tuberculosis, typhoid, cholera, malta fever etc.

The recent discovery of the sulphonamide derivatives and anti-biotics etc. has widely extended the field of chemotherapy and treatment of bacterial diseases has thereby undergone revolutionary changes.

Before entering into a discussion about the definition of the word 'chemotherapy' it is essential to understand some of the terms used by Ehrlich and to consider some of the salient features of the principles of chemotherapy which have a direct bearing on the experiments conducted.

In considering the principles of chemotherapy two main points will have to be taken into consideration. In the first place, the action of the chemical agent on the parasite should be considered. Some substances have a special affinity for a special kind of parasite and this Ehrlich designated as *parasitotropy* or *parasitotropism.* The special and selective affinity for the chemical agent may be confined to one species of parasite only—*monotropism,* or to several species—*polytropism.*

The second point which should be considered is the special affinity of chemical agents for the body cells. This Ehrlich termed as *'organotropy'* or *'organotropism'* and modern chemotherapy aims at finding substances which are highly parasitotropic, while possessing the least affinity towards the body cells, *i.e.,* very low organotropism. Diphtheria antitoxin is ideally suited to serve as an example of what a chemotherapeutic remedy should aim to be. It has a marked action on the *Cl. diphtheriœ* while it exerts little or no action on the body cells. It is, however, difficult to find chemical substances which will have a potent action on the parasites and at the same time have little or no action on the body cells.

Chemotherapy only requires the proportion of parasitotropism and organotropism to be present to such an extent as to allow the chemical agent to be useful in practical therapeutics. The organotropic effect may not be entirely absent, but it should not make the substance toxic and dangerous. To express the relation between organotropism and parasitotropism, Ehrlich coined the phrase *'chemotherapeutic index.'* It is the ratio between the maximum tolerated dose per kilo of body weight and the minimum dose per kilo of body weight required to destroy the parasites in the body. The higher is this ratio the greater is the applicability of the compound in the treatment of disease. Kolmer illustrates this ratio by a concrete example: Arsphenamine by intravenous injection is generally borne by white rats in doses of 0.120 gm per kilogram of body weight for an indefinite period; 0.140 may kill it in a few days. The former is therefore the MTD (maximum tolerated dose). When administered to rats infected with *T. equiperdum,* the smallest dose per kilo capable of completely eradicating the infection may be 0.004 gm, MED (minimum effective or curative dose). The results are expressed as follows:—

$$\text{Chemotherapeutic index:} - \frac{\text{Maximal tolerated dose per kg.}}{\text{Minimal curative dose per kg.}} = \frac{0.120}{0.004} = 30$$

This chemotherapeutic index, however, depends upon a number of factors, *e.g.,* the animal experimented upon, the strain of infective organism used, and therefore its value is not constant.

Various definitions of chemotherapy are given from time to time and Kolmer gives one which is used in the widest sense of the term and which includes both the prevention and treatment of a bacterial or protozoal disease with a chemical agent. "Chemotherapy

may be defined as the prevention or treatment of disease by chemical disinfection or inhibition of the parasitic causes without marked or serious toxic effects." It is that branch of the science of biochemistry which deals with the therapeutic properties of certain chemicals in the curative and prophylactic treatment of parasitic diseases.

The chemical agent may be natural or synthetic. Thus quinine is as much a chemotherapeutic agent as arsphenamine. Location of the parasite does not affect the definition in any way. The parasite may be in the blood, in the lymph, in internal organs or tissues or on the external surface (skin and mucous membrane). Thus the term chemotherapy in the modern sense includes the treatment of syphilis by arsphenamine, the treatment of hookworm by carbon tetrachloride, the treatment of pneumococcal infections with sulphonamide derivatives or the treatment of itch with sulphur. The only essential factor is the complete routing or crippling of the parasites, no matter what their nature and location may be, without seriously damaging the cells of the body.

2. Theories of Parasitotropism and Organotropism

(1) Side-Chain Theory

Ehrlich's theory.

Ehrlich applied the same theory to explain parasitotropism and organotropism as he did to explain the theory of immunity. Kolmer gives the following extract from Ehrlich's Harben lectures.

"I also wish to lay special stress upon my view that the drugs are attracted by and bound to the protoplasm molecule by certain atom groups. I am inclined to look upon this as somewhat analogous to the binding of the toxins and of similar protein bodies. Yet on the other hand, there are fundamental differences between the two. For, as I have always insisted, the mode of binding the toxins is peculiar in so far as it is the result of a certain kind of assimilation which obviously consists in a process of a more or less synthetic nature. These toxin-receptors which produce immunity are bodies of a more independent character, and appear to be especially destined for purposes of assimilation. This high degree of independence is evidenced by the fact that, in conformity with my side-chain theory, these receptors are very easily reproduced by the cell in excessive numbers, and after being separated from the cell, find their way into the blood.

Chemo-receptors.

"I have now formed the opinion that in like manner a part of the chemically defined substance is attached to the cell by groups corresponding to these receptacles; these atom groupings I will distinguish from the toxin receptors by the name of 'chemo-receptors.' This view is more practically supported by the fact that an atoxyl-fast strain of trypanosomes is also resistant to a number of substances related to atoxyl, but otherwise showing widely different chemical characteristics. Evidently, therefore, the arsenic acid radical here represents the point of attack which is common to this series of substances. There 'chemo-receptors' must, however, be regarded as of a simpler structure than the toxin receptors. They do not show a similar degree of independence, nor can they be thrown off into the blood. The number of such chemo-receptors for poisons which trypanosomes possess represents the number of points of attack. By means of the resistant strains we can count off, one by one, these groups that are open to attack."

This theory explains the affinity of a chemical agent or a drug for a parasite or a cell by supposing that there are certain special receptors on the cells to which these compounds become attached. Ehrlich's original work on the effect of various dyes and arsenicals on infective organisms, especially trypanosomes and spirochaetes, lent strong support to his hypothesis. According to him, a chemical agent cannot exert its action on the parasites, unless fixed by suitable chemo-receptors of the cells of the parasites. When arsenic or any other drug is administered, they are presumed to carry certain side-arms which are anchored to the special receptors of the cells of the organism. These side-arms have greater affinity for the receptors of the cells of the parasites than those of the tissues of the host, thus having a parasitotropic action with the least organotropic effect.

(2) Chemical Theory

Although little can be said against Ehrlich's well-supported hypothesis, Kolmer is inclined to believe that it has prevented a chemical theory being worked out properly, and absence of a well-directed research in this line has considerably curtailed the rapid advance of chemotherapy in modern scientific direction. He is of opinion that the phenomena of parasitotropism and organotropism are purely chemical or physico-chemical in nature. He mentions Meyer and Goethlieb's suggestion that arsenic produces its toxic effects by reacting with a substance which is present in the protoplasm in small amounts. Voegtlin and his co-workers have brought forward experimental data suggesting that the trivalent oxides

of arsenic are specific poisons for the SH group of glutathione and possibly other SH compounds in the protoplasm of the cell, with a consequent suppression of vital functions, which may explain the toxic and therapeutic effects of organic arsenicals like arsphenamin.

3. Factors Influencing the Action of Drugs

Attention has already been drawn to the different results obtained from *in vitro* and *in vivo* experiments. There is therefore some profound change occurring in the body which gives this divergence in the *in vitro* and *in vivo* experiments. The changes will mainly depend on the channels of absorption, for a variety of chemical and physico-chemical actions take place before the drug is distributed through the body to act on the parasites it is meant to destroy.

After oral administration, hydrolysis, emulsification, saponification, etc., occur in the gastro-intestinal tract before the drug is absorbed into the circulation. After absorption, the detoxicating properties of the internal organs, especially the liver, play an important part in the modification of the drug. The changes that occur after an intravenous injection are also important for, as the drug is directly introduced into the circulation, phenomena of lysis, agglutination, precipitation of proteins, etc., may occur. Important changes of oxidation and reduction may occur and a new compound may be evolved after combination with certain cellular constituents in the body.

After absorption the chemical agent is distributed in the body, not uniformly but selectively, and the aim of chemotherapy is to concentrate the agent in those tissues where the parasites are supposed to be numerous. The concentration in the tissues depends upon the method of administration, dose, elimination and the changes that occur in the body cells. Special affinity of a particular tissue will also determine the concentrations of the agent in the tissues.

Acquired or natural tolerance to the action of drugs is a well-known phenomenon. Certain individuals have got the power of tolerating large doses of poisonous drugs without showing toxic effects. In the case of habit-forming drugs, *e.g.,* alcohol, cocaine, nicotine, etc., the tolerance is probably psychic, developed as a result of the nervous system getting adapted to continued use. Other cases may be due to increased destruction of the poison, as is found with opium and its alkaloids, or the result of diminished absorption or increased elimination. Another conspicuous instance of tolerance is found in the formation of antitoxin. Such antitoxin formation is the result of repeated administration of foreign proteins, either animal or bacterial. Antitoxin formation has also been observed with certain glucosides, snake venom, poison ivy, etc.

4. Drug Resistance

(1) General Features

It was not, however, known till recent years that parasites too acquire tolerance to drugs. This tolerance in parasites to withstand the destructive action of a chemical agent is known as *drug fastness.* A vast amount of literature has since been piled up regarding the nature of drug fastness in various parasitic infections, such as trypanosomiasis, spirochaetosis, and from this standpoint the treatment of infectious diseases is of great interest in order to decide whether a particular remedy does or does not produce resistance in the causative organism. Franke and Roehl working in Ehrlichs laboratory recorded interesting observations on the drug fastness of trypanosomes. Working with nagana-infected mice they found that after feeding the mice with parafuchsin, the parasites disappeared from the peripheral blood. After sometime they reappeared, and by continuing the feeding it was observed that successive administration of the dye became less effective in removing the parasites from the peripheral circulation. Then a time was reached when the period of banishment of the parasites from the circulation became progressively shorter until the drug entirely failed to rid the blood of the organism. After transference of the strain to a healthy mouse it was found to be uninfluenced by the action of parafuchsin. The drug had therefore produced in the organism a heightened resistance to withstand the otherwise fatal concentrations of the same drug. This interesting discovery was soon followed by the further observation that trypanosomes could develop drug resistance against other drugs also. It is now known that nearly all the drugs which exert a chemotherapeutic action on trypanosomes can give rise to drug resistant strains. The important examples of these drugs, provoking drug resistance are:—(1) Aniline dyes. (2) Aromatic arsenic compounds. (3) Aromatic and non-aromatic antimony compounds. (4) Bayer 205. (5) Quinoline derivatives. (6) Guanidine compounds, etc. It should however, be noted that all these drugs even when closely related chemically do not

possess equal potentialities of making trypanosomes resistant to their action. In case of the aromatic arsenicals the drug resistance produced is not related to the arsenic content of the particular compound. From the data collected by various workers some generalisations have been worked out. Firstly it is held that a strain resistant to *an* aromatic arsenical or antimonial compound is also resistant to other aromatic antimonials or arsenicals. But the degree of resistance set up may not be the same quantitatively in each instance. Secondly, a strain resistant to aromatic arsenicals is sensitive to tartar emetic, but a strain resistant to aromatic antimonials remains resistant to aromatic aresenicals also.

The phenomenon of drug resistance has been observed by various workers both in experimental studies and clinically in the treatment of bacterial diseases. The nature of this resistance is only imperfectly understood but it is one of the most complex biological phenomena that has been observed in the reaction of the living organisms to the action of chemicals.

(2) The nature and mechanism of drug-resistance

It is very difficult to say at present why and how a certain strain of organism acquires the character of withstanding otherwise lethal doses of a poisonous drug. This resistance may be a character of the causative organism or it may reside in the tissues of the host. The idea that the resistance to the action of a drug is not a character of the infective organism but of the invaded host has been formulated. Such a theory is not tenable as it has been established that a strain of trypanosomes in mice made resistant to an aromatic arsenical compound is similarly resistant when transferred to rats or rabbits and *vice versa.*

The question now arises as to what this peculiarity can be attributed.

Voegtlin has devised a theory which postulates that trypanosomes acquire resistance to arsenic by virtue of the development of an excess of sulphhydryl compounds, which enable them to detoxicate the poisonous effect of arsenic. Kolmer also supports a similar theory and explains the phenomenon on the basis of a chemical interaction between the drug and the parasite. He believes that natural tolerance is due to the absence in the protoplasm of the cell of the parasite of a chemical constituent capable of interaction with the drug administered, or the presence of a substance which unites with the drug and renders it inert. Such a theory has, however, been criticised by Yorke (1931) who has shown that, contrary to the hypothesis of Voegtlin, the arsenic-resistant trypanosomes do not absorb any aromatic arsenicals even though these may be present in concentration rapidly fatal to the normal strain of parasites.

Some workers have recently adduced evidence which goes to support the view that resistant trypanosomes do not absorb the drug concerned. Similar observations have been recorded in the case of *Treponema recurrentis;* though the normal spirochaetes did take up gold and arsenic from solganal and neoarsphenamine respectively, yet, resistant forms did not absorb either of the drugs. It is believed that the phenomenon may be explained in a different way. The adoption of the various micro-organisms to changed environments may be the result of natural selection, *i.e.,* a gradual dying out of the more sensitive individuals and the survival of the more resistant ones.

Summing up the evidence by different workers it becomes clear that the exact mechanism of development of drug-resistance is still to be explained. It is quite probable that this phenomenon may ultimately be proved to be due to mutations in populations exposed to intensive selection over many generations. Whether these mutations are a spontaneous occurrence or a result of environmental factors also needs exploration.

(3) Drug resistance in trypanosomiasis, lieshmaniasis, Spirochaetosis, amoebiasis, malaria and bacteria

Trypanosomiasis.

In human trypanosomiasis the occurrence of drug resistance is a well-recognised interesting clinical observation. This phenomenon is more common in insufficiently treated patients who are given suboptimal doses of the drug concerned, thus affording chance to the trypanosomes to develop resistance against the drug. This drug resistance is said to persist indefinitely in case of some drugs, as for instance, it has been reported that resistance of trypanosomes against aromatic compounds of antimony, arsenic and acriflavine once developed, persists permanently. In case of other drugs however, such as Bayer 205, the resistant trypanosomes have been shown to have shed this acquired property completely during the course of about four years. Another important aspect of drug resistant trypanosomes is the fact that there are strong experimental reasons to believe that drug resistance even survives a passage through the insect host of trypanosomes, the Glossina. This raises

important public health issues as there is no doubt that insufficient dosage of aromatic arsenicals has produced quite a number of drug resistant strains of trypanosomes. These when taken by the Glossina and injected into another person would prejudice his chances of a cure as the particular strain received by this individual would react poorly to arsenical therapy.

Experimental data regarding drug-resistance on the part of *Leishmania donovani,* is meagre, Clinical observation, however, seems to justify the dogma that Kala-azar patients can be sharply divided into two categories on the basis of their response to treatment with pentavalent antimonials. A very large majority of patients fall into the class of ordinary cases, whereas the rest turn out to be resistants. A resistant patient has been defined as "one in which a cure is not effected by an ordinary course of treatment which will cure from 90 to 95 per cent of ordinary patients". More often than not, these resistant patients can ultimately be cured by means of repeated intensive courses of the usual treatment. In most instances the drug resistance is only relative rather than absolute.

The existence of drug resistant groups of spirochaets is not a very rare occurrence. In case of *Treponema pallidum, T. recurrentis, T. anserinum* and others, drug-resistance has been developed experimentally. *T. pallidum* becomes resistant to arsenicals relatively more easily than to bismuth or mercury compounds. The exact mechanism of the development of this resistance has not yet been finally elucidated. Instances have been recorded where syphilitics resistant to treatment had transmitted this feature along with their infection. On the other hand in cases of marital syphilis, instances are on record where one partner only was resistant to treatment, the other responding normally. Conclusion appears to be that drug-resistance in syphilis may either be a result of some change on the part of *T. pallidum* or due to some subjective peculiarity of the patient.

With regard to amoebic dysentery there has been a strong belief that emetine-resistant amoebae occur. Experimentally, strains of *Entamaeba hystolytica* have been produced, which are resistant to emetine. Inability to rid the patient of amoebae even after maximum doses of emetine hydrochloride has been recognised by many clinicians in the treatment of chronic ameobic dysentery. To what this failure may be attributed, it is difficult to state at present. The transformation of the vegetative parasites to the cystic forms, alternation in the tissues of the host affording a chance for the parasites to ward off the action of a chemical, the peculiar character of drug-resistance of the strain, all or some of these, may contribute to the results. It has, however, been observed that cases resistant to emetine react normally to carbarsone, yatren or to other non-alkaloidal amoebicidal remedies.

Resistant cases of malaria have also been described by several workers. Inability to prevent relapses in many cases of plasmodium infection has been ascribed by many to the resistance of the parasites to quinine.

Though quinine-resistant parasites may occur, the possibility of the development of this character in practical therapeutics seems to be small. Evidence available does not at present warrant the view that the failure of quinine treatment in some cases is due to the occurrence of quinine-resistant parasites. The mode and virulence of infection, the degree of susceptibility of the individual, the species and the geographical race of the parasites, are also to be taken into consideration in the alleged cases of failure with quinine. Faulty methods of administration and inadequate dosage may be responsible for many of the cases of failure. In addition, non-absorption of the alkaloid from the gastro-intestinal tract has also to be considered, before the existence of quinine-resistant parasites can be proved.

Drug resistance is also said to be encountered among bacterial organisms. It has not infrequently been met with sulphonamide drugs and even with antibiotics such as penicillin, Streptomycin.

5. The Reticulo-endothelial System

(1) Introduction and general

In recent years the reticulo-endothelial system has attracted a great deal of attention from workers interested in different branches of medical science. The anatomist, the physiologist, the pathologist, the hæmatologist and the immunologist have all evinced great interest in the study of this system and in the solution of one or more of its innumerable problems. The pharmacologist, who is interested in obtaining a knowledge of the mechanism of action of drugs, has also been directing his attention to this system in the hope that a study of it might be helpful in furnishing answers to

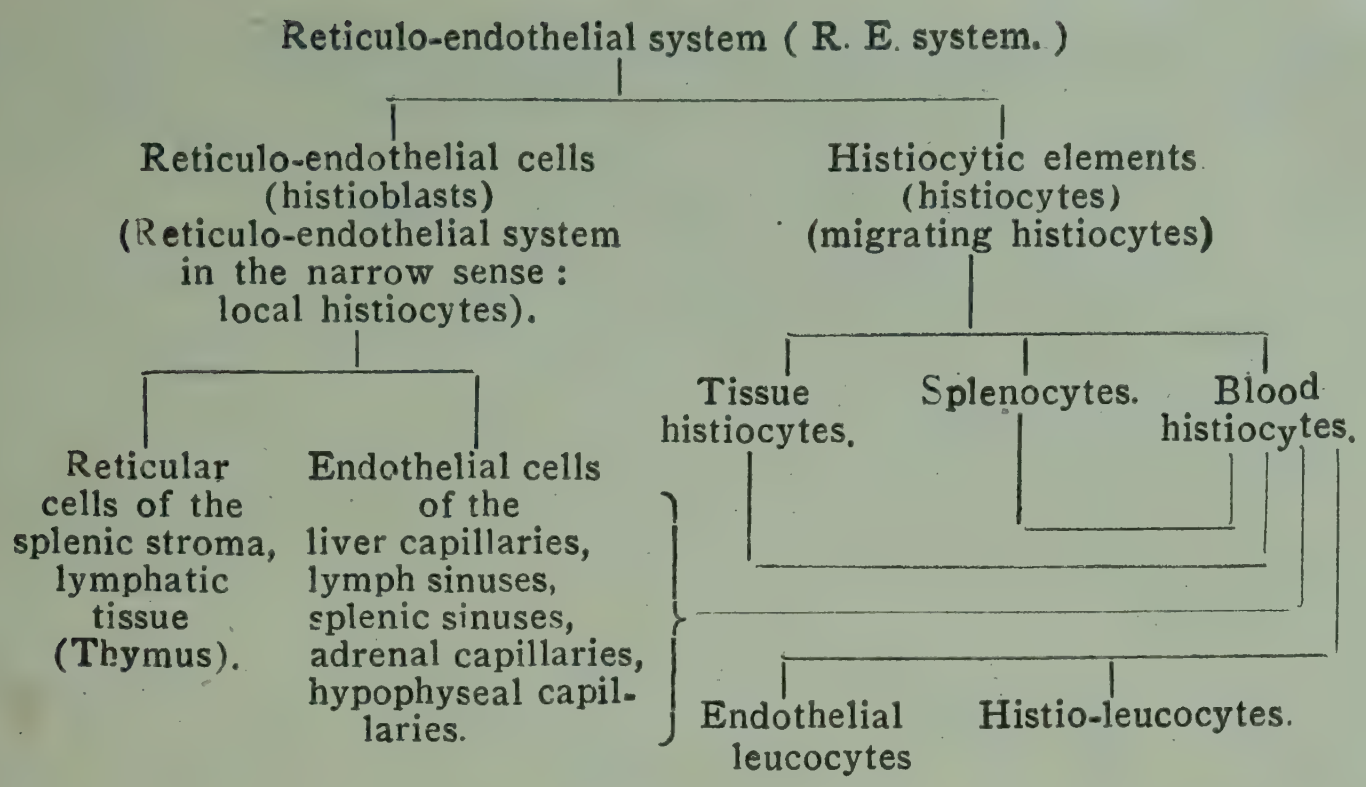
some of his problems. A perusal of the literature on the subject reveals that the efficacy of many drugs, including some of the so-called specific ones, may depend upon the functional efficiency of this system. The earlier view expressed by Ehrlich and others that the chemotherapeutic action of drugs depended entirely on their direct action on the causative agent of disease, has now few supporters. The accumulating evidence appears to be distinctly in favour of the view that drugs, in the majority of instances, act in an indirect way through the tissues of the body, and particularly through the cells of the reticulo-endothelial system. Time and further work alone can determine the exact role of this system in infection, immunity and chemotherapy. Incomplete though the knowledge may be at the present time, there seems to be very little doubt that the reticulo-endothelial system plays a part of great importance in recovery from diseases, more specially from those such as typhoid fever, tuberculosis, spirochætal infections, malaria, kala-azar and trypanosomiasis. In view of its increasing importance in tropical diseases, it seems necessary to give a brief description of the system, its accepted functions in health, and the part it plays in the cure of disease.

Widely scattered in the blood and tissues of man and other vertebrates, there are certain cells of mesenchymal origin and of the macrophage or large mono-nuclear type that possess the primitive capacity for phagocytosis and intracellular digestion. These take up all foreign particles that gain access to the body, whether they be inert or colloidal, inanimate or animate, bacterial or protozoal, and dispose of them in a manner best suited to the body economy. They collectively go by the name of the "reticulo-endothelial system."

The evolution of the reticulo-endothelial system is closely associated with the origin and development of parasitism and host-parasite adjustments. "In the long course of evolution of living things, it seems probable that the lower forms of life arose far in advance of the more highly differentiated forms. From the very moment of their first appearance upon this earth the latter had to compete for their place in nature with a vast number of microbial forms. In the course of the adjustments necessitated by this complex communal existence, various forms of parasitism were established. In many instances invasion of a host by a parasite was fraught with danger, and as a defence against it, mechanisms of protection of different degrees of efficiency were developed. Even in the most primitive forms of life one or more simple means of self-defence are discernable; as the needs for self-preservation become greater the mechanism of defence also becomes more complex." (W. W. C. Topley). In the unicellular animal the function of protection is performed by the cell itself and is merely an adaptation of the ordinary feeding mechanism. In lowly organised metazoa this function is relegated to certain special cells. In higher vertebrates and in man these cells of protection are very highly differentiated and are distributed widely throughout the body, and go by the name of the reticulo-endothelial system.

The history of the recognition of the cells of the reticulo-endothelial system may be said to have begun about the end of the nineteenth century. Mallory's 'endothelial leucocytes' and Metschnikoff's 'macrophage cells' are now recognised to be reticulo-endothelial cells and these workers, therefore, may well claim to be the first to recognise and point out the importance of these cells. Several subsequent workers also met with these cells under various circumstances and referred to them under different names. In 1913, Aschoff and Landau proposed to group together these differently named cells into a single system and to call it 'the reticulo-endothelial system.' In 1924, Aschoff gave a detailed description of the anatomical distribution of the cells composing the system and pointed out their functional unity and stressed their immunological importance.

The reticulo-endothelial cells have a very wide and scattered distribution throughout the body. They are to be found in organs, such as the spleen, liver, lymphoid tissue, bone-marrow, connective tissue, blood and endocrine glands. Amongst these organs, the spleen may be considered the largest store-house of these cells. The amount stored in the spleen as compared with other organs varies not only in different species of animals but also in different members of the same species. It has been found that after the removal of the spleen, regeneration of the reticulo-endothelial cells takes place more quickly and markedly in certain species of animals than in others. This has led to the presumption that the amount of reticulo-endothelial cells outside the spleen is also very variable and dependent upon the animal species. For the sake of clearness, the anatomical distribution of the cells of the reticulo-endothelial system as classified by Aschoff is given below in a tabular form.



Originally, Aschoff classified the cell elements of the reticulo-endothelial system into six groups according to the intensity of vital-staining and the size and compactness of the dye granules present in their cytoplasm. These groups are:—

(*a*) The *endothelial cells* of the blood and lymph vessels.
(*b*) The *fibrocytes* or ordinary connective tissue cells.
(*c*) The *reticulum cells* of the splenic pulp and lymphoid tissue.
(*d*) The blood *monocytes* and *splenocytes* [related to group (*e*) and (*f*).]
(*e*) The *reticulo-endothelial cells* lining the sinuses of the spleen, bone marrow, adrenal cortex and hypophysis.
(*f*) The *histiocytes* or the large phagocytic motile cells of the connective tissue.

Of these six types, the first and the second are the least phagocytic, the third and the fourth moderately phagocytic, and the fifth and sixth the most phagocytic.

The cells of the reticulo-endothelial system have been classified by Sabin into two main groups, namely, 'monocytes' and 'clasmatocytes.' The term 'histiocytes' is preferable to 'clasmatocytes.' Under normal conditions these two types of cells are found in all organs containing reticulo-endothelial cells. The ratio of monocytes to histiocytes in spleen puncture material is generally 6 to 1. In the peripheral blood, histiocytes are normally absent and the only representative of the reticulo-endothelial system is the monocyte. In pathological states and after experimental stimulation, histiocytes as well as all grades of intermediate forms have been found in the peripheral blood. These intermediate forms probably represent stimulated monocytes with increased phagocytic power. The reticulo-endothelial system has come to be recognised now as composed of two closely allied cell types. These cells differ in morphology and possibly in function. The histiocytes are said to play the chief role in the phagocytic mechanism of defence, while the monocytes are more closely associated with antibody production.

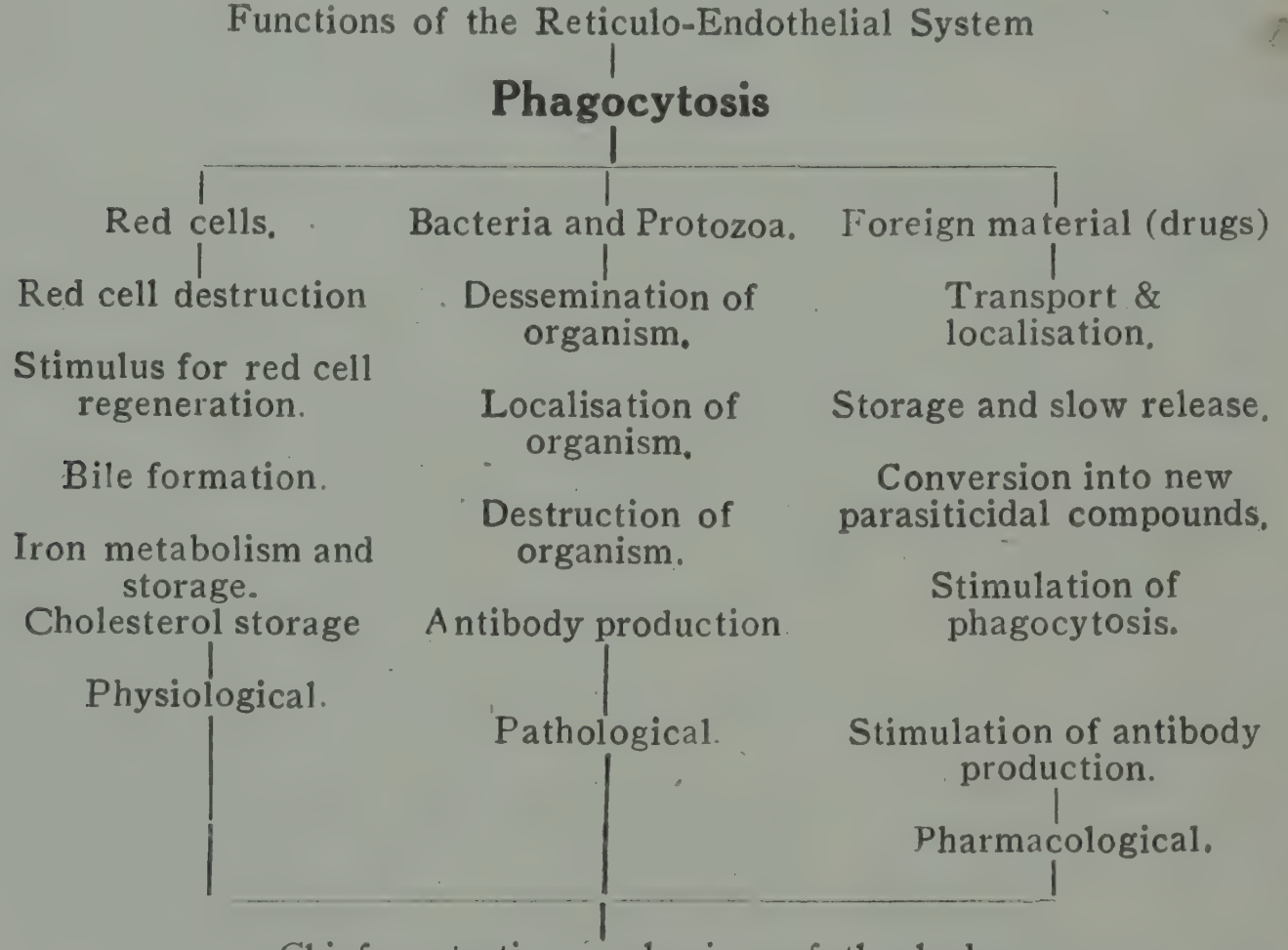
Normally, the two chief cell types composing the reticulo-endothelial system, as seen in supra-vital preparations, are quite distinct morphologically and can readily be distinguished from one another. Typical specimens of the two types may be seen in the blood of malarial cases twenty-four hours after an injection of quinine, or in the spleen juice of an advanced case of kala-azar prior to treatment. In these experimental and pathological conditions, some difficulty may be felt in classifying some of the forms intermediate between monocytes and histiocytes. With a little experience and discretion, this difficulty can easily be overcome. The following are some of the characteristics of the two cell types, in supra-vital preparations.

Monocyte is slightly larger than the lymphocyte, which it closely resembles. It has a big kidney-shaped nucleus with a deep and distinct indentation or 'hof' and shows the presence of a 'rosette' or clump of uniformly fine, neutral red particles at the 'hof' and a number of blue-staining mitochondria in its cytoplasm. Histiocyte is a much larger cell than the monocyte; its nucleus is very variable in shape and differs from that of the monocyte by being small in comparison to the size of the cell, by being often oval rather

than kidney-shaped and by occupying a more or less central rather than an eccentric position. The protoplasm of the histiocyte is somewhat granular, and mitochondria are generally absent, although occasionally, a cell may be seen with a few of them.

Functions of the Reticulo-endothelial System

The reticulo-endothelial system performs a multiplicity of important functions both in health and in disease. We already stated that phagocytosis is the chief characteristic of the cells of this system and to it we may add that it is this property that helps it to perform almost all its functions. The best way to understand how that is done will be by tracing the sequence of events as they take place after phagocytosis of different materials. The substances that these cells are known to ingest may be divided broadly into three main groups, namely (*a*) red cells, (*b*) organisms, such as bacteria and protozoa, and (*c*) foreign materials, such as drugs. For the sake of clearness the results of phagocytosis of these substances have been classified in a tabular form and then discussed separately in detail.



Phagocytosis

It is an accepted fact that the cells of the reticulo-endothelial system take up for purposes of destruction those red cells whose allotted span of life is over and also those that get damaged by inflammatory processes, toxins or parasites. In such diseases as malaria, kala-azar, typhoid fever, Weil's disease and poisoning by arseniuretted hydrogen in birds and phenyl hydrazine in dogs, phagocytosis of the damaged red cells can easily be observed. When hæmoglobin is injected into the circulation it is also taken up by these cells. *In vitro,* in tissue culture, the histiocytes have been observed to ingest red cells. Thus all observations made so far go to show that the histiocytes pick up old, damaged or dead red cells in order to destroy them. There appears to be no evidence, however, to suggest that they phagocytose healthy and young red cells.

Under normal conditions red cell destruction and regeneration are in a state of equilibrium and this helps to maintain the red cell count at a constant level. The organ that is chiefly responsible for the control of the equilibrium appears to be the reticulo-endothelial system. The stimulus for red cell production is probably furnished by the histiocytic elements after they have ingested and digested the red cells. It has been shown that when there is functional failure of histiocytes, blood destruction is not followed by regeneration to any appreciable extent. It is, therefore, probable that the reticulo-endothelial system provides the stimulus for regeneration of red cells and that in the absence of such stimulus the bone marrow fails to produce a sufficient number of red cells to make up the loss sustained.

McNee (1913) suggested that the reticulo-endothelial system as a whole is responsible for bile formation. Subsequent workers clearly established, by extirpation of the liver and other experiments, that bile pigment is formed in all tissues in which reticulo-endothelial cells are present, including Kupffer's cells in the liver, but not by the glandular cells of that organ.

One of the products of digestion of red cells and hæmoglobin is iron. There is increasing evidence to show that the reticulo-endothelial system is concerned in the metabolism and storage of this metal. In certain diseased states, such as pernicious anæmia, inanition of infants, toxic hæmolytic jaundice, malaria and after repeated injections of hæmoglobin, deposits of iron may be found in some of the cells of the reticulo-endothelial system. After splenectomy and experimental blockade of this system a marked disturbance of iron metabolism is often noticeable. It is highly probable, therefore, that the system is of importance in the storage and metabolism of this metal.

Storage of cholesterol appears to be another function of this system. Whenever there occurs an excess of cholesterol in the blood, either as a result of experimental cholesterol feeding or in certain diseases, such as diabetes, lipæmia, some cases of icterus and contracted kidney, the excess of cholesterol is taken up by the histiocytic elements of the liver, spleen and skin, and may be seen infiltrating these cells. The peculiar skin tumours at times seen in diabetics, (Xanthoma diabeticorum) are nothing but collections of histiocytes loaded with cholesterol esters and allied substances.

(2) Phagocytosis of Parasitic Organisms

Bacteria.

It was shown long ago that bacteria injected intravenously are taken up largely by the endothelial cells of the spleen to the liver and the lungs. There is also proof of phagocytosis of organisms in many infectious diseases. Everyone is familiar with the persence of different bacterial organisms within macrophage cells in films of inflammatory exudates. In infections diseases such as pneumonia, typhoid fever, bacillary dysentery, tuberculosis, leprosy, actinomycosis and spirochætal diseases, the reticulo-endothelial cells may actually be seen loaded with these organisms in infected tissues. In all these diseases and in the granulomatous diseases, in particular, the characteristic cellular reaction is a proliferation and multiplication of the reticulo-endothelial cells, and the tubercle, the leproma and the gumma, are all formed as a result of this specific cellular immunity reaction.

Protozoa.

There is considerable evidence to show that the blood-inhabiting protozoa are also phagocytosed by the cells of the reticulo-endothelial system, macrophage cells take up malarial parasites in birds; red cells and malarial parasites have been found within the large mononuclear cells. Despite this, there are some who still believe that the cells of the reticulo-endothelial system take up only dead or dying malarial parasites. Recent observations in connection with the role of the reticulo-endothelial system in malaria have shown that the cells of the reticulo-endothelial system phagocytose living as well as dead malarial parasites. In kala-azar, oriental sore and dermal leishmaniasis *Leishmania* are mostly found within the cells of the reitculo-endothelial system. Experimentally, when *L. donovani* are injected into susceptible animals, irrespective of their route of entry, subcutaneous, percutaneous, intravenous, intrapleural or intraperitoneal, they are picked up by the cells of the reticulo-endothelial system and carried to the spleen, liver and bone marrow. There they live and multiply within these cells. In trypanosome infections, although phagocytosis of the parasites has been observed, it does not appear to be a very pronounced feature. As regards phagocytosis of other protozoa by the reticulo-endothelial cells there is very little evidence. Taking the available data into consideration, we may say that the cells of the reticulo-endothelial system are capable of phagocytosing many of the blood-inhabiting protozoa.

There is no evidence that the cells of the reticulo-endothelial system phagocytose helminthic worms that gain access to the blood or tissue. It is probable that they take up antigenic substances liberated from the worms, either during their life or after their death and disintegration, and lead to the production of antibody. What part this antibody plays in overcoming the helminthic infection it is not possible to say and as such the role of the reticulo-endothelial system in diseases due to worms still remains a mystery.

Having shown that phagocytosis of bacteria and protozoa by reticulo-endothelial cells does occur, we may consider next the fate of the ingested organisms. Are they destroyed within the cells or do they live and multiply there? Both in the case of bacteria and protozoa, phagocytosis is not always followed by death of the ingested organisms. While it is true that large numbers of them are killed by these cells, there is enough evidence

to show that some of the organisms survive and even multiply within these cells. In the case of leishmania and hepatozoon infections, the parasites actually live and multiply within the reticulo-endothelial cells. It has been shown that certain bacterial organisms are found alive within phagocytes and that the latter at times afford the bacteria protection from the injurious effects of serum antibodies in their environment. It is clear that phagocytosis and destruction are two separate functions of the cell and need not necessarily be present together at one and the same time. Depending upon the functional state of the cell, the nature of the organism ingested, and certain other factors, death may or may not occur after phagocytosis. It has been shown that one of the factors that helps to destroy parasites effectively is specific antibody. Although the importance and value of antibodies in different infectious diseases vary, being probably high in virus and certain bacterial infections, moderate in protozoal infections, and slight in helminthic infections, there is evidence that antibodies do sensitise all parasites in such a way that other destructive processes, such as phagocytosis and lysis, can carry on their functions more successfully.

Antibody production.

The site of formation of antibodies has been a disputed question for a considerable time. Years ago Metchnikoff suggested that the macrophages secrete some kind of ferment which helps them to destroy the parasites they ingest. Later, this view was given up, and the belief arose that antibody was elaborated by all tissues locally, at the site of inoculation of antigen. Recently, a large number of investigators studied this question and their work strongly indicates that formation of antibody takes place only in the reticulo-endothelial system. Splenectomy experiments are all in favour of this view. The most suggestive evidence, however, in this connection has been obtained by tissue culture work. It is evident that an antigenic substance irrespective of its source or nature introduced into the animal tissue is taken up by the cells of the reticulo-endothelial system and removed to organs, such as the spleen and liver, where they stimulate the production of suitable antibody. Antibacterial, antitoxic and lytic antibody are all formed according to requirements.

Phagocytosis of foreign material.

The chief characteristic of the reticulo-endothelial system that led to its discovery is phagocytosis of foreign substances. There is an enormous amount of evidence to show that its cells readily ingest substances such as vital dyes, carbon, and Indian ink particles. If, for instance, one of these substances is injected intravenously into a rabbit and then films from spleen, liver and lung are examined, particles of the injected substances can be detected within the reticulo-endothelial cells. While little difficulty is experienced in demonstrating the presence of inert and coloured particles, such as those mentioned above, the detection of soluble chemical substances (drugs) that are generally used in the treatment of disease, is a difficult matter. By the use of microchemical tests and special staining methods, colloidal and organic preparations of metals, such as iron, silver, antimony, bismuth and arsenic, have been shown to be present within the reticulo-endothelial cells. As regards the finding of alkaloidal and other drugs, *e.g.,* quinine within these cells, there is very little evidence; it is possible that they are also taken up by these cells and removed to the spleen, bone marrow, suprarenals and kidney.

Regarding the disposal of particular substances after phagocytosis, there is evidence that the reticulo-endothelial cells in the course of their wandering, transport them to various organs and tissues (lung, liver, spleen, bone marrow and kidney), where they are either eliminated or stored. Very little is known about the fate of soluble chemical substances probably a considerable quantity is excreted within a short time through the normal channels of elimination and only a very small quantity is found in the body, possibly in the reticulo-endothelial cells. Here they are present either in their original state or in an altered form. As most of these foreign substances gain access to the body as drugs, at a time when it is in a diseased state, the question of prime importance is how they bring about cure and beneficial results and what part the reticulo-endothelial system plays in it.

(3) Relation to Chemotherapy

Introduction.

It is unfortunate that despite a large amount of experimental work very little progress has been made in the understanding of the mode of action of drugs in diseased states or of the role of the reticulo-endothelial system in pharmacotherapy. In the case of protozoal infections we, possess knowledge about the specific action of some drugs. Being of far bigger size than bacteria and more easily demonstrable in the blood and tissues by direct examination of smears we can get a true picture of the effect of treatment on protozoa and on the course of infection, and we can also correlate these findings with various cytological and serological changes. This has enabled workers to tackle several

of the problems in chemotherapy through investigations on protozoal infections, such as malaria, kala-azar and trypanosomiasis. As regards helminthic infections the hindrances to progress have also been many and our knowledge of the mode of action of anthelmintic drugs is not very satisfactory. Nevertheless, taking the evidence as a whole, there appears to be very little doubt that chemotherapeutic action of drugs in general is not merely a simple direct action on the parasites. It may be true that in certain helminthic diseases, and possibly also in some protozoal infections, the action of specific drugs is predominantly a direct one, but with regard to the majority of other remedies, there is absolutely no evidence that they have any marked parasiticidal effect. It has repeatedly been shown that parasites are actually more vulnerable to drugs when they are brought into contact with them within the body than outside it. Futhermore, drugs are rapidly excreted from the body and the dilution in which they occur in the blood and tissues after their administration in therapeutic doses is so very high that simple direct action alone can hardly be said to account for their efficiency. Blockade and splenectomy experiments have clearly established that dysfunction of the reticulo-endothelial system reduces and at times completely abolishes the therapeutic value of drugs. In view of these and other observations the consensus of opinion is that the reticulo-endothelial system plays an important part in chemotherapy.

It would appear that in bringing about cure, the direct action of drugs on parasites is of the greatest importance in helminthic infestations; of moderate importance in protozoal and bacterial infections; and of least importance in most of the virus diseases. As regards the importance of the reticulo-endothelial system in overcoming infections, the reverse order appears to be the most likely, *i.e.*, the system plays a part of slight importance in helminthic diseases, of moderate importance in protozoal and bacterial diseases, of the highest importance in virus diseases. How far these views are correct time alone can decide.

Mode of action.

The different ways in which the reticulo-endothelial system can possibly help drugs in bringing about a cure are:—(1) That it acts as a store-house for the drug and releases it slowly as required—thereby preventing rapid elimination and ensuring continuous supply. (2) That it carries the drug to the neighbourhood of lesions, where it is most needed. (3) That it elaborates new compounds from the drug with greater parasiticidal power. (4) That it is stimulated by the presence of the drug in circulation and thereby its functions of phagocytosis and antibody production become more pronounced and effective. In support of the above view the following evidence may be cited. It has been shown that when germanin (Bayer 206) was injected in a basis of 6 per cent agar, its efficiency was greater on account of the slow rate of absorption of the drug from the agar which acted as a 'second spleen.' This supports the first contention. After injections of antimony compounds into monkeys, the highest concentration of the drug was found in the liver, where there was also a concentration of infection with *Leishmania*. It has also been experimentally shown that the reticulo-endothelial cells localise certain colloidal substances, especially those that carry a negative electric charge in inflamed tissue in preference to other tissues. This establishes the second contention. A study of the action of arsenic compounds in splenectomised and nonsplenectomised animals suggests that the reticulo-endothelial system is probably concerned in the conversion of these compounds into more efficient germicidal substances. This corroborates the third contention.

The phagocytic function of the cells of the reticulo-endothelial system is stimulated by quinine in malaria and this is said to be partly responsible for the cure of the disease. The antibody formation is said to be increased after antimony treatment of kala-azar and that this probably is responsible for cure. These observations lend support to the fourth contention.

Further evidences in support of the above can be obtained from a discussion of the mode of action of some of the important drugs used in the treatment of different infectious diseases. The following is a brief reference to some of them:—

Role in helminthic infections.

The role of the reticulo-endothelial system in the chemotherapy of anthelmintic drugs is not fully understood. Although the evidence is strongly in favour of the mode of action of these drugs being a direct one on the worms themselves, there are suggestions in certain cases, that the beneficial results obtained may be attributable, in part at least, to an indirect action, which is dependent upon the functional efficiency of the reticulo-endothelial system. This view appears to be supported, partly at least, by the results of treatment of helminthic infections of the blood and tissues. The dermal tests reported to be useful in the diagnosis of some helminthic infections, such as schistosomiasis, hydatid diesase, trichiuriasis, filariasis and intestinal worms, are evidence of sensitisation to antigenic substances from the worms, and are most likely due to sessile antibodies

elaborated by the reticulo-endothelial system. If it is so, then the system may be presumed to play some part in the cure of these infections. But at present we are not in a position to understand what exactly is the role of these antibodies in helminthic diseases.

In protozoal infections.

The role of the reticulo-endothelial system in the treatment of protozoal infections has been extensively studied. Although there are several drugs which may be called specifics and which are known to act indirctly through the reticulo-endothelial system, the mode of action of a few of the more important ones alone is discussed below.

In action of Emetine.

While there is evidence to show that the therapeutic efficiency of this drug in human amœbiasis is probably due to a direct action of the alkaloid upon *E. histolytica,* the work of Dale and Dobell clearly suggests "that emetine and the amœba are not only factors to be considered in the cure of dysentery and that the missing factor is probably the interaction between emetine and the host's tissues." If this is correct, then what other tissue of the host can play this important part more successfully than the ubiquitous reticulo-endothelial system? Available evidence does not suggest the way in which the system enhances or ensures the efficiency of the drug.

In action of Quinine.

The mode of action of quinine in malaria has been studied by different workers, but the views regarding it are still very varied. While some believe that the drug attacks the parasites directly, others claim that it acts in an indirect manner through the host's tissues. It has been shown that quinine, by accelerating the natural processes of mobilisation, proliferation and functional activation of phagocytic large mononuclear cells, brings about rapid engulfment and ultimate destruction of the malarial parasites. Whenever the drug fails to elicit the suitable reticulo-endothelial response, no improvement is noticeable, and after splenectomy of monkeys infected with *P. knowlesi* quinine fails to bring about the same degree of beneficial results as when the organ is present. It has been recently (1944) shown by congo red intra-venous injection method that quinine in therapeutic doses increases the efficiency of the reticulo-endothelial system and in unchecked malaria this system is depressed. When malaria infection is duly checked by quinine it is stimulated. These results leave no doubt that the reticulo-endothelial system plays a part in the cure of malaria by quinine.

Action of antimony.

The mechanism by which antimony destroys *Leishmania donovani* and brings about cure of kala-azar is still unknown. It is very doubtful that it is a direct parasiticidal action. It has been shown that tartar emetic has no action on *Leishmania donovani* cultures in *vitro,* and that *Leishmania donovani* grows as luxuriantly in NNN tubes, to which neostibosan and aminostiburea are added, as in control NNN tubes. When antimony compounds are injected intravenously, they are excreted rapidly in the urine the dilution in which antimony is present during the short period of its stay in the circulation, is not very high, and no direct action on the parasites can possibly be conceived of, in such low dilutions. The opinion that its action is through the tissues of the body is, therefore, fast gaining strength. Acton and Chopra (1927), and Chopra and Das Gupta (1929), found that when antimony compounds are injected intravenously into kala-azar patients, it causes enlargement and rhythmic contractions of the liver and spleen and that this is due to an increased functional activity of the adrenals. This observation led them to suggest that the alteration in the permeability of the vascular walls brought about by the hyper-activity of the adrenals caused a diminution in the permeability of the cells of the reticulo-endothelial system and this in turn led to the starvation and death of the leishmania parasites present in them. These findings have been confirmed in an indirect manner by studying the changes in the leucocyte picture after adrenalin injection into kala-azar cases, before, during, and after, treatment. This work clearly indicated that a condition of hypoadrenia existed in kala-azar cases and that treatment helped to bring about a normal condition. But what part this alteration in the adrenalin content played in overcoming the infection could not be clearly determined. Certain cytological studies on kala-azar patients with the help of supravital staining technique showed the importance of the reticulo-endothelial system in infection and immunity in kala-azar. It was found that within the animal body, *Leishmania donovani* are present only within histiocytes and that in untreated kala-azar cases there is an enormous increase in the histiocytic elements of the reticulo-endothelial system and a reversal of the normal monocyte-histiocyte ratio. During and after treatment a distinct drop in the number of histiocytes is noticeable and this is followed by a gradual restoration of the monocyte-histiocyte ratio to normal. Whenever antimony failed to bring about these cellular changes, the patient showed no clinical improvement.

In action of Arsenicals.

Various compounds of arsenic are used in the treatment of trypanosomiasis and spirochaetal infections. The mode of action of these preparations seems to be an indirect one, like that of antimony compounds. The presence of arsenic within the reticulo-endothelial cells has actually been demonstrated by the silver impregnation method. Splenectomy experiments in spirochaetal infections have demonstrated that infections held

in check in animals break out into acute manifestations in splenectomised animals and that treatment of these with specific drugs is not as effective as in non-splenectomised animals. There is evidence to show that the mortality rate is increased and the cure rate greatly decreased after splenectomy and that an intact reticulo-endothelial system is necessary for the full therapeutic activity of compounds of arsenic. From these and other observations it may be suggested that the reticulo-endothelial system functions. (*a*) by serving as a depot for concentrating arsenical drugs, (*b*) by helping in the production of more efficient parasiticidal substances and (*c*) by increasing the amount of antibody produced and by bringing about destruction of parasites.

In Bacterial and virus infections.

As regards bacterial infections, in acute forms the role of the reticulo-endothelial system in the action of potent chemotherapeutic substances discovered during recent years is not clearly understood. Possibly they act in the same way as they do in protozoal diseases. For virus diseases no drugs have so far been discovered that have any marked specific action on the causative organism. Our knowledge of chemotherapeutic drugs used in these diseases is very unsatisfactory. The beneficial results that are obtained in virus diseases are chiefly dependent upon the natural resources of the body to combat disease. The best we can do in these infections is to help the natural process at work, in one way or another. This has been attempted with a certain degree of success.

In the case of chronic infections, a large number of protein substances such as peptone, milk, muscle extract, vaccines and sera, and certain colloidal metals have been used to stimulate the natural responses of the body. Although our knowledge as to how these substances act is still very vague, yet there are reasons to believe that the therapeutic results obtained are probably attributable to a non-specific stimulation of the reticulo-endothelial system by these substances. It has been shown that serological alterations such as increase in titre of antibodies, and cellular changes such as leucocytosis and increased phagocytic activity of mononuclear cells, are noticeable after injections of these non-specific protein substances. There is evidence that these substances are concentrated in organs such as the liver, spleen and bone marrow; it is likely that through these organs an increase in the efficiency of the natural immune processes occurs.

The action of specific anti-sera used in the treatment of acute infections is chiefly a direct one upon the causative agent of disease. They either destroy the agents themselves or prepare them in such a way that other mechanisms of protection may destroy them subsequently. Specific vaccines, when injected into the body, are probably picked up by the reticulo-endothelial cells and result in the formation of specific antibodies which act as described above.

(4) Conclusion

Conclusion.

In conclusion we may state that evidence obtained from studies on the mode of action of drugs in infectious diseases taken as a whole, strongly suggests that in the overcoming of infections the reticulo-endothelial system plays a part of no mean importance. The exact manner in which the system responds to the stimulus of treatment appears to depend upon the nature of the infective agent concerned. While certain parasites are readily destroyed by phagocytes, others require to be disposed of by the destructive action of lytic anti-bodies. The ideal response in the first case is a mobilisation and functional activation of the phagocytic cells of the reticulo-endothelial system, whereas in the second instance the best results are obtainable by increased antibody production. When either of these methods fails, the reticulo-endothelial system attempts to utilise other methods at its command, such as elaboration of powerful parasiticidal substances from the drugs used. From this there is every reason to believe that beneficial results of drugs are not due to one single factor but rather to a combination of factors. Which one of these factors plays the predominant part varies in each case. The specific drug is that which is capable of stimulating the natural processes concerned in the cure of disease, and in addition bringing about such changes by direct and indirect action on the parasite or its environment, as would be conducive to the success of the natural processes at work. If this is so, then it is easy to understand that a knowledge of the reticulo-endothelial system, which is the chief protective mechanism of the body, is essential not only for the proper understanding of infectious diseases, but also for the carrying out of successful treatment.

CHAPTER V

STIMULANTS AND TONICS

GENERAL STIMULANTS—CARDIAC AND VASOMOTOR STIMULANTS—RESPIRATORY STIMULANTS.

Stimulant and Tonic Drugs

The word 'tonic' is commonly used by physicians and by the laity at large to denote remedies which stimulate the protoplasmic activity of the tissues, thereby increasing the genenal metabolic processes of the body. Tonics are generally employed in conditions of asthenia, neurasthenia and general depression of the body activities after illness, strain and effects of climatic conditions. In tropical climates one frequently meets with cases who are victims of chronic maladies or have become debilitated by living in a hot and damp atmosphere. Good nutritious food and rest, combined with tonic drugs, go a long way towards curing this condition. Tonics produce a sense of wellbeing, increased strength and vitality by stimulating the functional activity of the digestive organs as well as by improving the general condition of the hœmopoietic system. They contribute towards the improvement of the general tone of the rundown body or some of its component parts. Tonics are, therefore, classed as general tonics, digestive tonics, cardiac tonics, hæmatinics, nervine or nerve tonics and so on. It should be realised, however, that the convalescent from disease improve more by adequate rest, proper selection of nutritious and easily digestible diet and good hygienic surroundings than by tonic drugs alone, which only aid and speed up the process.

1. General Stimulants

The drugs classed as stimulants or tonics are very large in number, but here it will suffice to discuss only a few of the important ones.

Bitters.

Bitters are always prescribed to patients convalescing after long protracted illness, where there is a definite derangement of the digestive functions and an upset of the general metabolic processes of the body. They are used to improve the appetite of such patients and are generally given before a meal. They produce a reflex flow of the gastric juice which is beneficial to the impoverished digestive process. The effect of these drugs is never due to direct contact of the drug with the oxyntic cells of the glands in the gastric mucosa, but is entirely reflex through the gustatory nerves. The common bitter substances in use are gentian, quassia and calumba, while many of the bitter alkaloids, such as the cinchona alkaloids and strychnine, are commonly employed for their bitter action in small doses.

The preparations of cinchona and quinine are employed in very small doses to stimulate the gastric mucosa; they thereby increase secretion, improve appetite and aid digestive processes. The infusion of cinchona is an excellent stomachic in cases of mild gastric catarrh and atonic dyspepsia. The compound tincture in combination with nux vomica is often used as a general tonic and appetiser. In all post-febrile states, in convalescence from prolonged illness, in general debility and cachexias from various chronic maladies, quinine is often prescribed as a tonic in conjunction with nux vomica, iron and arsenic. Though quinine is believed to be a cardiac depressant, the drug in small doses acts as a sedative and by slowing down the pulse improves the irregularity in rhythm and is thus beneficial to the heart.

Nux Vomica preparations.

Preparations of nux vomica are often prescribed for their simple bitter action. They invoke a reflex flow of the gastric juice and tone up the muscular

wall of the viscus, thereby improving the appetite and aiding the digestive functions of the patient generally. Strychnine is used in atonic dyspepsia and during convalescence from acute illness. Besides its bitter properties, strychnine is often used as a tonic to the muscular system. It diminishes the 'synaptic resistance' in the nervous system, whereby a smaller stimulus can pass a greater number of synapses than before and bring into action more nerve fibres. It has, therefore, been used in post-diphtheritic paralysis of muscles and post-operative paralysis of the stomach and gut. It is a good tonic for sexual debility, impotence and spermatorrhœa. Strychnine is often reputed as a cardiac tonic, but views are very conflicting regarding its direct effect on the heart. Experiments show that the drug has no direct effect on an isolated perfused heart; in fact, it depresses it. It is, however, held that the drug does indirectly improve the heart beat through its stimulating effect on the medullary centres. The drug is undoubtedly an emergent cardiac tonic, as when injected with atropine in threatening cardiac failure, it will almost immediately raise the tone of the organ and strengthen the pulse. Injections of strychnine are useful in weakness of the heart in acute fevers, such as penumonia, influenza and diphtheria. It is held that the drug increases the blood pressure by its effect on the medullary centres and in this way improves the nutrition of the myocardium by improving the coronary circulation. Strychnine is also a useful respiratory stimulant in chronic bronchitis, pneumonia and emphysema. Strychnine is a tonic in the real sense of the word, in that it increases the tone of all striped and unstriped muscle tissue in the body which is lowered after disease and other debilitating conditions.

Alcohol.

Alcohol has long enjoyed the reputation of being a good stimulant. It is often used in cases of chronic debility and various wasting diseases. It has been shown that regular use of moderate doses of alcohol with meals promotes digestion, increases appetite, checks waste, favours deposition of fat and so alcohol practically possesses all the properties of a true food. It is a food in the sense that it is readily oxidised, yielding considerable energy, which is only available for immediate emergency use and cannot be stored as a reserve for future use.

Alcohol excites secretion of gastric juice and it can also produce a direct stimulant action on the fundus of the stomach, causing an abundant secretion of dilute gastric juice. Apart from being a digestive stimulant, it is also used as a restorative in urgent cases of fainting or threatening cardiac failure due to shock, hæmorrhage, etc. Here it acts as a diffusible stimulant reflexly and thereby increases the pulse rate, blood pressure and respiration.

Alcohol is contained in various tonic wines sold in the market and is often prescribed for convalescing patients. It should always be given in small and frequently repeated doses as given in this way, it is completely metabolised. If larger quantities of alcohol are taken, its toxic effect over-balance any of the beneficial effects produced.

Thyroid.

The use of endocrines as tonics is of recent origin. They appear to form a system which regulates the rate of metabolism, and the growth and development of the body. Thyroid is reputed to be a stimulant of the endocrine glands. It has been considered as the trigger gland of the body, which sets other endocrines to action. Besides its use in thyroid deficiency diseases, it is often prescribed as a tonic in neurasthenia. It is useful to convalescing patients and to fatigued subjects after long-continued strain and over-work. In tropics, due to the enervating influence of the climate, such cases are frequently met

with and the drug in small doses has given encouraging results. It acts by increasing the rate at which energy is produced by the body. Administration of thyroid adds more fuel to the fire of metabolism in the body and the oxygen intake rises. The body-machine is geared at a higher level, and food normally used to supply the current demand for energy and stored as fat, is now used to yield more energy and only a small proportion is reserved for future use. Appetite is thus increased, patients feel more energetic and the gland now serves the purpose of a tonic in the right sense of the word. Thyroid B. P. known as thyroid siccum should be prescribed in doses of ½ to 1 grain twice daily for this purpose for a period not exceeding a fortnight at a time.

Arsenic.

Arsenic is believed to be a digestive stimulant when given in small doses (1/50 gr). It has been shown by experiment that arsenic accelerates formation of red blood cells, but the effect is slight and unimportant. Inorganic compounds have probably no marked therapeutic effects and should not be prescribed.

Calcium.

Calcium compounds have a reputation as a stimulant. Calcium is a normal constituent of the body tissues and is indispensable to the life of all organisms. The calcium content of the body is about 2 per cent of its entire weight. In pulmonary tuberculosis calcium injections are said to be useful but there is no rational foundation for that belief. Even in urticaria, serum sickness and chilblains, its efficacy is doubtful though it is largely prescribed. Calcium lactophosphate and glycerophosphate with cod-liver oil and ultra-violet therapy are useful in rickets and malnutrition.

Iron.

Iron is a haematinic. It is a natural constituent of the body. The adult human body contains 3 to 5 grains of iron in the form of haemoglobin; the liver contains 0.15 to 0.25 gm. There is no large store-house of iron in the body which can be called upon to supply a deficiency, though it is believed that the liver and spleen can store considerable amounts. From 0.5 to 1 mgm of iron is lost daily in the urine, and 5 to 20 mgm in the fæces. The amount of iron in an average mixed diet is 5 to 20 mgm a day; it is therefore very little more than is necessary to make good the normal daily loss. Iron is only prescribed when there is deficiency as in anæmias. The tone of the nervous system is improved with iron therapy in cases of mental overwork, neurasthenia and neuralgia, because it stimulates the body metabolism. In combination with arsenic, strychnine and quinine, it is prescribed with benefit in all forms of debility with or without anæmia, and where there is a general lack of tone in the tissues. In all conditions where there is loss of appetite and sluggishness of body functions, iron therapy is beneficial.

Phosphorus.

Phosphorus is the normal constituent of most tissues. Its popularity is a relic of the notion that it was a brain food as it occurs in a high proportion in the brain tissues. The lactophosphates, glycerophosphates and hypophosphites often prescribed as tonics, are inactive.

2. Cardiac and Vasomotor Stimulants

Physiological considerations.

The circulatory system with the lungs may be regarded as a single functional system which supplies oxygen to the tissues and removes carbon dioxide from them. They are efficiently controlled according to the needs of the body from time to time by a central and a local mechanism. The heart forms the central and the most important organ in the system and continues functioning regularly and efficiently throughout life without ever resting. Normally, in a healthy adult, it makes on an average about 103,680 contractions a day, though these considerably vary in different stages of life and in diseases. The capacity of muscular exertion of an individual in health and the fate of the patient in disease, all depend on the condition of the heart. The heart is characterised by its unique properties—namely, automaticity or stimulus production, rhythmicity, excitability, contractility tonicity and conductivity.

The heart has the first call of blood leaving it by the aorta and as much as 20 per cent of the total output may enter the coronary circulation. Unlike voluntary muscles, heart can never enter into oxygen debt. Hence a deficiency in oxygen supply to its need will result in an accumulation of lactic acid definitely depressing and injuring the efficacy of this vital organ. A deficient coronary circulation will impoverish heart from lack of proper nutrition and the whole body will share the misery.

The heart has great reserve power to meet the demands of emergencies and utilises only a fraction of it for its work, when the body is at complete rest. It has been found that the oxygen consumption during complete bodily rest is 1/4 litre per minute, a heavy meal increases to ½ litre a minute and walking at the rate of three miles an hour increases it to 1 litre.

The action of the heart is regulated by two sets of nerves, namely, the sympathetic and the vagus. The sympathetic is the augmentor nerve of the heart, it increases the force of contraction of both the auricles and the ventricles, and the rate of conduction from auricle to ventricle. The vagus is the inhibitor nerve of the heart, it slows the heart, depresses the force of beat of the auricles and also the rate of conduction from the auricle to the ventricle. The vagus has a constant control over the activity of the heart; the control is slight at two extremes of life and maximum in early adult life. The vagus also constricts the coronary arteries and the sympathetic dilates them. In spite of the regular and constant nervous control over the heart's activities, the pace maker of heart has a natural rhythm, irrespective of any nervous control.

The demand of the tissues for oxygen varies from minute to minute, and this is regulated by the blood flowing through the small arteries and the arterioles. The distribution of the blood is so efficiently made that the organs, whose continuous activity is most essential to the maintenance of life, receive the major share most promptly and regularly. Arrest of circulation in the brain only for a few seconds will result in unconsciousness. The brain regulates the general supply of the blood to other organs after ensuring a rich supply for itself. The body mechanism is so adjusted that the demand for blood supply is met in emergent cases for some particular functioning organ at the expense of the other. This is met with by a proper regulation of the arterioles mostly by a central and a peripheral mechanism. The arterioles are very richly supplied with vasoconstrictor nerves and by their action as well as by the vagal and vasomotor centres in the medulla, the general blood pressure and the supply of blood to different organs of the body are determined. The arterioles supplying the most vital organs of the body, the constant activity of which is most essential to life, are not supplied by the vasoconstrictor nerves. The arterioles terminate in thin-walled capillaries, which perform rhythmic contractions. At rest, only a small number of them dilate to carry on the circulation to distant parts, while when an organ functionates, the arterioles supplying it dilate and a greater number of capillaries open, and the blood flow to the organ increases manifold. The normal circulation of the blood depends on the maintenance of a certain amount of tone in the capillaries and the veins, failing which the blood stagnates and the heart does not receive adequate quantity of blood to contract upon and to carry on its proper function.

The splanchnic vessels are richly furnished with vasoconstrictor nerves, and next come those supplying the skin and the muscles. It is stated that the blood flow through the skin and muscles at rest is 12-10 ccm, per 100 gm, per minute, liver and kidney 70 ccm, brain 150 ccm, and the endocrines 600 ccm.

Heart-failure.

The failure of the heart to maintain an efficient circulation in the body is not an uncommon complication in diseases. From the clinical point of view the following types of cardiac failures are met with:

(1) Acute cardiac failure causing sudden death; it occurs in angina pectoris, ventricular fibrillation and coronary disease. Acute failure is also produced by chloroform, lightning or electrical shock.

(2) Sub-acute cardiac failure occurs in acute infectious diseases, *e.g.*, pneumonia, typhoid, diphtheria, influenza, etc.

(3) Chronic cardiac failure usually occurs in chronic valvular disease and myocardial lesions.

In considering the treatment of heart-failure it should be remembered that the essential cause of heart-failure lies in the heart muscle.

Treatment.

The treatment of heart-failure may be conveniently described under the following heads:

When the heart is compensated, careful instructions should be given to the patient regarding his mode of life. It is of vital importance that he should have abundance of physical and mental rest in his daily life. Excitement, anxiety, worry and emotional strain should, as far as possible, be avoided. He should not have any physical exertion that would give rise to breathlessness, palpitation, fatigue, a sense of tightness across the chest, or precordial pain. At the same time a certain amount of graduated exercise is desirable. Walking on the plains is preferable to hill-climbing. When the cardiac affection is progressive, the amount of exertion must be correspondingly reduced. With regard to diet, the food should be nutritious, easily assimilable and not likely to cause indigestion. The fluid intake should be restricted. A carbohydrate diet is bulky and apt to cause flatulence, while a high protein diet increases the resistance in the peripheral circulation. The diet should, therefore, be a mixed one with predominance of the albuminous element. A careful search should be made for a septic focus, which should, if possible, be eradicated. The bowels should be kept open and regular.

General management.

Drugs.

The underlying idea of treatment with drugs should be, (*a*) to give rest to the heart as much as possible, (*b*) to improve the nutrition of the heart, (*c*) to increase the force of systole and prolong diastole, thus improving the efficiency of the contractions and lengthening the period of rest. There is a great deal of confusion as to what drugs should be classed as true stimulants to the heart. The word 'cardiac tonic' usually conveys the suggestion that an increased activity or tone is being brought on slowly, but which is more enduring, while 'cardiac stimulant' means a prompt but transient increase in the activity of the heart which is not enduring The idea of treatment of heart-disease is not to increase the frequency of a failing heart, because thereby the process of exhaustion is hastened. During the compensated stage, no cardiac tonics are required.

Digitalis.

Digitalis is the most useful drug for failing compensation. In the words of Mackenzie, "Digitalis, acting on the vagus, pulls the reins of the heart, acting on the heart muscle it is a most useful whip, at the same time providing it with food by improving the circulation. By slowing the heart it makes it regular and also this gives the heart an opportunity of resting, so secondarily improving the contractility, conductivity and excitability of the organ."

Lewis writes, "The giving of digitalis to unselected heart cases is much to be deplored. Those who regard digitalis as a cardiac stimulant, mistake its character; its chief action is to rest the heart. To the heart, foxglove is not tonic, but powerfully hypnotic. It controls the diastole of the heart: it extends the period of ventricular sleep."

There are two conditions in which digitalis is indicated: (1) Heart-failure, specially of the congestive type, with cardiac dilatation, diminished contractility and tonicity of the heart muscle, anuria and anasarca. Mitral cases, with water-logging, and cases of auricular fibrillation, with a ventricular rate exceeding 80 per minute, react better than aortic cases. (2) Presence of a chronic auricular fibrillation with tachycardia, where heart-failure has not supervened. Such cases may be kept going and heart-failure postponed indefinitely by adequate digitalis medication directed to maintain the ventricular rate below 80 per minute at rest. Provided congestive heart-failure is present, hypertension and aortic regurgitation are not contraindications, as was formerly taught. The results, however, in the above two conditions, as well as in agina pectoris, are not always as favourable as in the mitral group.

Patients put on digitalis for the first time, should go to bed and remain here until the reaction to the drug has been investigated fully. As a routine, the tincture of digitalis, biologically standardized, is used. A usual and safe dosage for an adult is 60 to 75 minims of the tincture daily, by mouth. But in India and warm climate leaf powder should be preferred. The tincture may be given divided into two or three doses, diluted with water, and not mixed with other medicines.

Digitalization.

According to Eggleston the average amount of the drug required to digitalise the human heart is 9/40 grain of the powdered digitalis or 2¼ minims of the tincture per pound body weight. In estimating the weight, the amount of fat and œdema must not be taken into account. In non-urgent cases, one-fourth of the calculated dose is given at once, one-fourth after six hours, and thereafter one-tenth to one-eighth of the total every six hours. But in urgent cases, one-third to one-half is given at once, six hours later one-fifth to one-fourth and after another six hours one-eighth to one-sixth.

This means that the amount of active standardized digitalis preparations that would be necessary by mouth to produce full therapeutic effects within 36 to 48 hours in an adult who has not received any digitalis within ten days is about 1.5 gm (.=1.2 gm USP XI) of powdered leaf or 15 ccm (12 ccm USP XI) of the tincture. One half or one third of the total dose is given at once and the remainder two portions after an interval of four to six hours in urgent cases. If digitalis has been taken within the previous fortnight it is best to give 0.2 gm (0.16 USP XI) of powdered leaf three or four times a day, the patient being carefully observed for toxic effects before a subsequent dose is given. It usually takes 6 to 8 hours for a single dose to take effect.

The Eggleston method of heavy dosage and rapid digitalization is reserved for the cases 'in extremis' and should be adopted only where the patient can be examined at least twice every day. Ordinarily, the daily dose of 60 minims of the standardized tincture should be continued until either the desired slowing of the heart rate is obtained, or, until nausea or diarrhœa appears.

Digitalis lanata.

In recent years *Digitalis lanata* has come into use. It is a potent source of glucosides, stated to be nearly 4 times as potent as *D. purpurea. D. lanata* contains four glucosides, of which the chief is lanadigin. It is only one-third as toxic as strophanthin; it accumulates in the body more than strophanthin, but much less than the purpurea glucosides. Clinically, it is said to act more rapidly than any of the purpurea preparations, and the nature of action is similar.

For interavenous medication digoxin BP is best. Digoxin is pure glucoside isolated from the leaves of *digitalis lanata.* It is a pure glucoside of constant composition and is slightly soluble in water. The drug is marketed in ampoules each containing 0.5 mgm in 1 ccm of 70 per cent alcohol. This is mixed with 10 ccm of normal saline and given intravenously slowly at least 2 minutes being taken. Full effect occurs in 2 hours, after which the dose may be safely repeated if the ventricular rate has not been slowed enough. The initial dose should be 0.5 or 1 mgm. The maintainance dose is 0.5 mgm a day orally.

Strophanthin.

Strophanthin has the same action as digitalis, the standard tincture of strophanthus (dose 2 to 5 min.) acts in ½ to 1 hours, but the effects are unreliable and it is often ill-tolerated. Strophanthin B.P. is given in doses of 0.25 to 1 mgm or 1/240 to 1/200 grain intravenously. The initial dose of 1 mgm is roughly equivalent to about 6 ccm of the B.P. tincture of digitalis. When the urgency has passed off, digitalis can be given by the mouth. Caution has, how-

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ever, to be exercised, as the drug sometimes behaves in an unaccountable manner, loss of consciousness has followed an ordinary dose in a weakened patient. It should always be well diluted with 10 ccm of normal saline, and injected, very slowly, into the vein. In acute febrile heart-failure, sudden death has rarely occurred with intravenous strophanthin.

Camphor and its derivatives.

Camphor is now-a-days largely used in the treatment of heart-failure, especially in chronic myocarditis with simple cardiac insufficiency. In typhoid and pneumonia it is considered as a valuable stimulant to the heart. With regard to its effect on the heart, no decisive and accepted proof has been obtained of any direct action on the organ. Given subcutaneously, camphor acts as a local irritant and reflexly stimulates the medullary centres. The evidence available suggests that it does not directly stimulate the heart, but like alcohol causes redistribution of the blood, which will benefit the patient generally and the heart-beat particularly. It is now generally employed in doses of one to one and a half grains, dissolved in 1 ccm of ether or olive oil, and injected hypodermically.

Niketha mide.

Niketha mide is popularly known under the proprietary names of coramine, nikamide and anacardone; cycliton is an allied drug. For injection, niketha-mide B.P. is used as 25 per cent solution in water. Nikethamide is absorbed if given orally, but stimulatory effects are best obtained when given intravenously. Besides its general stimulating effects on the central nervous system it has a marked stimulating effect on the respiratory centre. There seems to be little support for the claim that in repeated doses it is of value in the treatment of myorcardial diseases.

Leptazol.

Proprietary names of leptazol are cardiazol, phrenazol, hexazol, triazol, azoman. Metrazol is the official name in the U.S.P. Injections of leptazol B.P. is a 10 per cent solution in water; dose 5 ccm to 10 ccm. Leptazol is absorbed when given by the mouth. The effective dose in narcotic poisoning is from 3 to 5 ccm of the injection given initially; the dose is increased until the patient responds or convulsions are produced.

Caffeine.

Caffeine is a direct cardiac stimulant acting on the excito-motor area of the heart. It produces an increase of the pulse rate, but from the point of view of rational treatment, stimulation of the heart alone will be of little value unless the circulation is improved as well; its efficacy is, therefore, doubted. Caffeine sodium salicylate or caffeine sodium benzoate in doses of 2 grains may be injected subcutaneously to produce stimulation of the heart. But this action is of relatively little value, because in a failing heart, the increase in frequency, leads to exhaustion.

Adrenalin.

Adrenalin is a peripheral sympathetic stimulant. It augments and accelerates the cardiac beats and is one of the most potent therapeutic agents to resuscitate a failing heart. Adrenalin, when given hypodermically or intravenously, especially by the latter route, causes vaso-constriction and raises the blood pres-sure, and consequently in damaged myocardium it may throw a sudden heavy strain on the heart and thereby do positive harm. The action of adrenalin is transient and, except in conditions where there is low blood pressure with feeble heartbeat, no permanent improvement in circulation is brought about. It has, however, been used in threatened or complete arrest of the heart, when that is not due to fibrillation. Intravenously, adrenalin acts almost immediately. The dose should be about one-fiftieth of that of the hypodermic dose, and there is no remedy available that bears comparison with it in such cases. Intracardiac adrenalin to resuscitate the patient in cases of arrested heart-beat or in anæsthetic

syncopy, has been tried. The dose of adrenaline by the subcutaneous route is ½ ccm or less. It is stated that 3 to 5 minims is the optimum and effective dose even in severe spasms of asthma; higher doses than ½ ccm cause shock, rigor, and profound bodily tremors and depression for many hours after. For intravenous purposes, a dose of 2 minims diluted with 2 ccm of distilled water is given very slowly. Under no circumstances should adrenaline be used in ordinary cardiac failure, as it puts extra strain on the heart by increasing peripheral resistance by vaso-constriction.

Adrenalin is very unstable in neutral or alkaline solutions and in such solutions it is very readily oxidised to form inert substances. The solution turns brownish with age and the deterioration in potency is proportional to the discoloration and any solution thus discoloured is unsuitable for use.

Ephedrine

The vaso-pressor effect of Ephedrine is now well recognised. It is used by some clinicians to guard against heart-failure in pneumonia and influenza; ¼ to ½ grain three times daily is given in cases of low blood pressure. The advantages of ephedrine over adrenalin are that it can be given by the mouth and produces an action of much longer duration. The toxic effects of the drug should, however, be borne in mind.

Chopra and co-workers had shown that pseudo-ephedrine has a direct stimulant action on the myocardium. A tincture made from ephedra, which contains both ephedrine and pseudo-ephedrine, has been used with success in epidemic dropsy, pneumonia, diphtheria, etc.; the usual dose is 15 to 20 mins. thrice daily.

Glucose. It is a valuable agent in heart-failure. When given intravenously after leeching or venesection, it acts as a food to the heart and stimulates the function of the kindneys. The details of therapy are given elsewhere.

Cane-sugar is considered to be a valuable cardiac nutrient. In the body, it is first converted into glycogen and thence to glucose, and as such serves as a source of food for the heart muscle in conditions of prolonged strain in some acute fevers, as pneumonia, influenza, typhoid as well as in cases of chronic cardiac failure. The average adult dose for the sugar is 4-8 ounces a day.

Oxygen. It has been found very useful in cardiac disorders with hypertension, arteriosclerosis, aortic insufficiency, etc. It is administered in form of oxygen bath, 90° to 95°F. (32°—35°C). It lowers the blood pressure, and there is a coincident diminution in the size of the heart. These effects persist only a few hours after the first bath, but after 15 to 20 baths, very prolonged and even permanent physiological and therapeutic effects are obtained. They are contra-indicated where the blood pressure is much below normal, especially if associated with mitral defect and profound anæmia.

3. Respiratory Stimulants

Carbon dioxide.

Carbon dioxide is a respiratory stimulant *par excellence.* Addition of 1 per cent of CO_2 to the inspired air increases the volume of air breathed per minute by 25 per cent, 3 per cent CO_2 increases it 100 per cent, and 6 per cent increases it from 500 to 600 per cent. Carbon dioxide, however, should not be used indiscriminately and like other potent remedies may prove a dangerous weapon if used without reasonable care. Lower respiratory centres are not stimulated by CO_2 and therefore under certain conditions harmful effects rather than beneficial may result by increasing the concentration of CO_2 in the inspired air.

Oxygen.

Inhalation of oxygen containing 7 per cent CO_2 acts as the most powerful respiratory stimulant and is the method of choice in a variety of conditions—such as drowning, CO poisoning, in apnœic new born infants and in morphine poisoning. If the respiration has been totally arrested inhalation of this mixture should be forced by artificial respiration. Prolonged periods of unconciousness following anaesthesia, injury or poisoning with narcotics and abdominal injuries which inhibit the cough reflex lead to blocking of bronchi with mucous plugs. This may be followed by atelectasis or massive collapse and then pneumonia. For the prevention of such conditions inhalation of a mixture of above proportions is most valuable and is recommended to be used as a routine measure after prolonged operations.

Caffiene coramine, etc.

All general nervous stimulants such as caffeine, strychnine, coramine and cardiazol stimulate the respiratory centre and are particularly indicated, when the respiratory centre becomes insensitive to rise of CO_2 tension in the blood. Coramine and cardiazol are very popular in treatment of patients in extremis. Caffeine is a very strong respiratory stimulant, distinct increase in the rate of respiration and volume of air breathed and a clear diminution in the pressure of CO_2 in the alveolar air following its injection.

Peripheral stimulation.

Peripheral stimulation by several means such as flicking with a wet towel (especially used for apnœic new born or morphine poisoning) chloroform inhalation, injection of camphor etc. also excite the respiratory centre.

CHAPTER VI

PAIN AND INSOMNIA

INSOMNIA AND THE USE OF HYPNOTICS—HYPNOTIC AND ANALGESIC DRUGS—PAIN AND ITS CAUSATION—OPIUM DERIVATIVES AND OTHER ANALGESICS.

1. Insomnia and the Use of Hypnotics

Aetiology of sleep.

Sleep is one of the primary necessities of human life. It is a natural state which normally comes on when an animal ceases to attend to external or internal stimuli, the reflex activity of the central nervous system is inhibited to a degree which is normally accompanied by unconciousness. Falling asleep is the natural consequence of keeping awake.

Loss of sleep or prolonged deprivation, leads to many pathological changes in the body. Normal sleep is a protective mechanism by which the physiological wear and tear of the body are made good. It appears at definite times and if unsatisfied, will pass off; but frequent abstinence leads to permanent derangement of body function. Prolonged sleeplessness causes various pathological changes, such as histological changes in the cerebral cortex and probably the appearance of a toxin in the blood.

Sleep is a condition of unconsciousness to surroundings. There are many theories as to the etiology of sleep. Muscular relaxations, rise in the intra-cranial pressure, cerebral anæmia and similar other theories have been advanced. Cerebral anæmia will undoubtedly produce unconsciousness and sleep, but the natural sleep is not, however, attended with any material alteration in the cerebral circulation. Knowledge regarding the mechanism of sleep is still very scanty. The nervous system acts as a whole. Pathological and experimental lesions in the infundibular region produce pathological sleep. A diencephalic sleep centre has been described but the term waking centre would be more appropriate. Presence of sleep suggests an abeyance of the katabolic activity associated with the sympathetic system and an excess of the anabolic activity associated with the parasympathetic system. The diencephalic centre is stimulated by sensory impulses from many directions. Insomnia might be due to excessive cortical activity, and for this there is no need to use drugs strong enough to depress the diencephalon.

Others regard sleep as a rhythmic depression of the activity of the brain and insomnia as due to one or a combination of two causes: abnormal afferent stimuli affecting the brain at a time when its depression is due or increased excitability of the brain cells. The rational treatment of the first type is to remove the stimuli if possible. Increased excitability may be congenital or acquired; there are brains which are easily kept awake from childhood. Excitability can be increased artificially, as by caffeine, ephedrine and high blood pressure. Bromides are especially valuable for normal brains kept awake by such stimuli as worry.

Common causes.

The common causes of not sleeping are:—(1) Pain which can be relieved by 1/4 gr of morphine. (2) Mental and emotional excitement or excitement caused by moderate doses of narcotic drugs. (3) Dread of non-sleeping which is cured by restoring the confidence of the patient. (4) The no-sleep habit, which should be corrected.

There are different types of insomnia with different causes and treatment for one type may not be suitable for another. Some persons are light sleepers from their infancy; in others the ability to sleep has been lost from overwork, sorrow or worry or after a severe illness or nervous breakdown. Women may get insomnia when they get sleepless nights in bringing up children from nightly waking. Some individuals do not sleep when they go to bed, while others fall asleep and wake up with a start and gradually lose the desire to sleep. Others awake between 2 and 5 in the morning or awake and drowse throughout the night. Still others are more or less asleep all night but are not sufficiently rested because they are tossing about or have bad dreams.

The commonest cause of sleeplessness is mental fatigue and muscular or nervous tension, the brain remaining active. This is particularly the case when intense mental work is being done in the course of the evening. Many people start worrying about not getting sleep when they go to bed. They are so much afraid of insomnia that they keep themselves awake. Some live in noisy streets and cannot sleep on that account.

If a person suddenly develops insomnia without any apparent cause think of hyperthyroidism or encephalitis. In other persons it may be due to arteriosclerotic injury to the brain. Psychopathic individuals heading for a nervous breakdown do not sleep for fear of night-mares.

Treatment.

The treatment of insomnia is of importance. Many of the chronic invalidating diseases, such as chronic malaria, kala-azar, ankylostomiasis are accompanied by loss of sleep and the practitioners are confronted with the problem of procuring rest for the body, in addition to finding some specific remedy for the malady.. The use of hypnotics is not, however, always indicated and treatment may be described under two heads.

For minor ailments, such as flatulence and distension of the stomach no hypnotic drugs are called for, careful regulation of the dietary together with some gastric sedative will relieve the condition and help to produce sleep. In cases where the specific cause of sleeplessness is untraceable, a careful survey has to be made as regards any unusual mental stress or strain which may be responsible for the loss of sleep.

General measures.

General hygienic measures, regular and careful dieting, exercise in the open air without involving any physical fatigue, will be effective in those cases where insomnia is due to overwork and prolonged mental exertion. A holiday in the country, in the hills, or at the seaside are often the most successful remedy, although some hypnotic may be necessary some time or other to supplement the effect. Besides conditions that favour relaxation, *viz.*, a quiet room, and a suitable bed will be of great help.

There can be no real cure for insomnia unless the patient can reduce the hours of mental work especially night work. When insomnia is bad it may be wise to go to bed an hour earlier than it is intended to sleep. During the first few minutes after the light is turned out, all thinking about problems, worries and activities should be stopped and mind concentrated on going to sleep. Every bit of will power will be needed to keep out of mind all disturbing thoughts which produce tension. The mind should be filled with thought which relieve tension. Listening to or reading an unexciting book may be helpful. The physician should impress on the patient that insomnia is nothing terrible; there are thousands of people who enjoy good health and work hard who have not slept for years. The patients may possibly be sleeping on a chair for hours and imagine they have slept only for a very short time.

Patients with indigestion sleep better if they take a very light meal at night. Some people sleep if they take a warm drink before retiring. Alcohol is often an excellent sedative and induces sleep, especially in older persons. Older people who have to get up at night to urinate should cut down the amount of liquid in the afternoon and evening.

Drugs.

Drugs should be used when necessary without hesitation if person is likely to suffer from the effects of insomnia next day. Habituation though it occurs is met with in a class of indisciplined people. If after a few minutes the person feels that his brain is very active and that sleep will not come, and if stress of next days work make it essential, a rapidly acting barbiturate (sodium luminal ½ to 2 gr) may be taken. These drugs do not produce euphoria like opium derivatives and a habit is less likely to be formed. The physician should have a thorough knowledge of action of sedative drugs and should prescribe the drug best suited for a particular case when it is essential. In psychopathic persons, barbiturates are ineffective and produce terrifying night-mares.

2. Hypnotic and Analgesic Drugs

A hypnotic is a narcotic drug which is used to enable the patient to get the sleep which is due to him, or desired by him.

Ideal hypnotic.

According to Clark the properties of an ideal hypnotic are:—(1) The drug must produce a reliable hypnotic effect. (2) The hypnotic action must be produced without a preliminary stage of excitement. (3) The drug must not irritate the stomach. (4) The drug must be absorbed readily, so that the hypnotic action is produced at a regular interval after the administration of the drug. (5) The drug must either be broken down in the body or else be excreted rapidly, so that it produces no after effects next day, and does not produce cumulative poisoning when taken daily. (6) The drug must not produce dangerous side actions, such as cardiac depression. (7) There must be a sufficient margin of safety between the dose required to produce hypnosis and that which produces medullary depression. (8) The drug must not produce tolerance, nor a drug habit, when given regularly over long periods.

There is no drug, which possesses all these qualities together and it is only after prolonged use that some of the disadvantages are found out. Drugs of low toxicity include the inorganic bromides (*e.g.,* sodium, potassium, ammonium, strontium, and calcium bromides), alcohol, phenazone and its derivatives, aspirin and salicylic acid derivatives, barbituric acid derivatives, etc.

Analgesics might produce sleep by reducing afferent stimuli and can be usefully combined with hypnotics, but a fixed inflexible combination is dangerous. A small analgesic dose of morphine combined with chloral might be more effective than a large dose of the either drug alone. Combinations of hypnotics acting at different points are also useful. Salicylates have a special property of diminishing painful stimuli and thus act secondarily as hypnotics.

The abuse of hypnotics. Serious dangers in connection with the use of hypnotic drugs are well known. They are liable to produce habit formation and the patient may be unable to sleep without his customary dose. Addiction to the drug may result and this is more serious as increasing doses are necessary to produce the desired effect.

Automatism.

Automatism may result from a small dose dulling the patients intelligence and he may forget that he has already taken a dose and may only remember his intention to do so. In this way patients have been known to empty a whole tube of tablets left by their bed side, with consequent disasterous results. If such a person awakes at all he may have no memory of his performance.

The following are some of the hypnotic drugs which are commonly used:—

1. *Alcohol.* The effect obtained is proportional to the patient's previous abstinence; a strict tee-totaller falling to sleep with a small dose whereas a habituee requires much larger doses. For a patient unaccustomed to alcohol, a mild hypnotic dose is about 20 to 30 ccm equivalant to about 1 to 2 ounces of whisky or brandy.

2. *Chloral hydrate.* The hypnotic effect is produced with doses of 10-20 grains by the mouth in ten to fifteen minutes' time and sound sleep in an hour. It lasts several hours resembling the normal physiological sleep and the patient can be easily and completely aroused and awaken refreshed without any depressant after-effects. It may also be given per rectum. The dangers of depression of the heart and respiration with chloral are overrated and it can be considered a safe hypnotic if it does not overstep the therapeutic limit.

The average poisonous dose of chloral is about 90 grains, but death has been recorded after 30 grains. The safety margin of chloral is, therefore, variable. The drug should not be used for control of convulsions or for any condition requiring the full narcotic dose of about 75 grains.

Action of chloral-hydrate.

The action of chloral resembles barbitone and the sleep usually comes on peacefully and without any unpleasant side effects. The effect of a big dose of chloral, however, does not persist so long as a big dose of a stable barbiturate, chloral being more rapidly destroyed in the tissues. The effect of barbitone and phenobarbitone may last for more than 24 hours but that of chloral for 12 to 18 hours. Chloral cannot be kept in form of compressed tablets which is a disadvantage, but it is a mild rapidly acting hypnotic. It is often given in combination with opium and bromides for restlessness in early stages of acute fevers, such as, pneumonia. The *three fifteens* is a popular draught containing 15 minims of tincture of opium, 15 grains of chloral hydrate and 15 grains of potassium

bromide. In painful illnesses where the brain is excited by worry or toxins of fever it prevents sleeplessness.

Use for infants.

Chloral is the safest drug to be used as a sedative in infants (during teething) and small children, an effective dose being 1 grain for each year of age. Chloral addiction is rare. Drugs allied to chloral are butylchloralhydrate and chloralamide (or formamide) which are not in the B.P. Chlorbutol is a B.P. drug which has some local and anaesthetic action on nerve endings as well on the brain. The dose of all three is 15 to 20 grains.

Combination of chloral and camphor forms a liquid paste which evaporates when applied to the skin and has a pain relieving effect.

3. *Paraldehyde.* It produces natural sleep in 15 to 20 minutes lasting for several hours; but the effect of the drug is less certain than chloral hydrate and it has no analgesic action. It is said to produce no depressing effects on the heart and respiration in therapeutic doses and can, therefore, be safely administered in cases complicated with heart disease. The disadvantages of the drug is that it has got a very unpleasant taste which, however, can be rectified by giving it in some flavouring agent. Prolonged use of paraldehyde leads to irritation of the throat and stomach, an unpleasant odour in the breath, dizziness and faintness, and a habit not unfrequently develops.

The dose of paraldehyde for single administration is one drachm (4 ccm) and in some cases two drachms may be given. In cases where the drug has to be given repeatedly, one teaspoonful every hour should be given till sleep is produced.

Paraldehyde dissolves in 9 parts of water. It is given floated on top of some water or some strong tasting substance such as liquorice extract to disguise its nasty burning taste. It freezes at about 52°F and forms crystals.

Action of paraldehyde.

Paraldehyde when taken by the mouth is rapidly absorbed and rapidly excreted mainly by the urine but partly by the lungs. Death has occured with two ounces but such doses are not always fatal. With 60 to 120 minims (4 to 12 ccm) unconsciousness may be produced in an adult in 5 to 15 minutes. For sometime the patient may be difficult to arouse and he may go on sleeping for some hours if he is in need of sleep. If he is in pain he may remain half asleep and half awake. Paraldehyde is prescribed in painful toxaemias such as that of pneumonia. It should be combined with an analgesic like morphine.

Paraldehyde is prescribed in delerium tremens as it may send the patient to sleep and because of its nasty taste it is not likely to form a habit. It is used as a basal narcotic in doses of one drachm (4 ccm) per stone of body weight.

Avertin.

4. *Tribromoethyl alcohol.* This is a white crystalline powder unstable in air. Bromethol B.P. goes under the proprietary name of *Avertin.* It is a solution of 1.0 gm of tribromoethyl alcohol in 1 ccm of amylene hydrate. It decomposes in presence of light giving off hydrobromic acid which is an intense irritant. Acidity of solution should be tested before use and if the pH is higher than 3.0 the solution should not be used.

Dosage.

The drug is moderately rapidly broken down in the liver. When given per rectum, maximum effect is attained in about 30 minutes. The dose should be proportionate to the body weight varying from 0.06 gm to 0.12 gm per kilo, according to the degree of the effect desired. No matter how heavy the patient, more than 10 gm should not be given as a single dose. It is not recommended per rectum. Intravenous injections produce a profound narcosis. In prepairing solutions for rectal injection the temperature should not be raised more than 50°C (122°F) as it decomposes and produces severe ulceration.

Toxic effects are rare though necrosis of liver has been observed after repeated administration in tetanus. As a basal narcotic it is safer than barbiturates but less safe than paraldehyde.

5. *Sulphone Group.* The drugs belonging to this group, sluphonal, trional and tetronal are insoluble, slowly absorbed and slowly excreted and dangerously cumulative producing hematoporphyrinuria. These have been entirely discarded.

6. *The Barbiturates.* Although urea has no narcotic effect rearrangement of atoms in its molecule produces narcotic properties. Barbituric acid is formed by combination of urea and malonic acid. It is unstable and has no narcotic action. Addition of various side chains to its molecules, however, confers stability and narcotic properties to its compounds.

The mode of action of all barbiturates is the same, the difference being only in dosage. The persistence of effect depends on the stability of the compound which again depends on the rate at which the side-chains undergo oxidation. The kidneys excrete the stable barbiturates, in disease of this organ excretion is diminished and narcotic action may be prolonged. The oxidation of unstable barbiturates taken place in the liver and in the disease of this organ these compounds are more, dangerous than usually; they may produce prolonged narcosis and death. Even stable barbiturates may be decomposed by the liver in small quantities. *Mode of action.*

In case of unstable barbiturates a little tolerance is acquired but in case of stable compounds a fair amount of tolerance is acquired, this power probably depending on the increased power of the liver to oxidize these drugs. Tolerance is only slowly acquired and, therefore, moderate repeated doses may produce cumulative results. *Tolerance.*

Barbituric and allied acids are poorly soluble in water but their sodium salts are soluble and are administered by injection. Barbiturates have no local irritant effect or remote toxic effects on the viscera. Instances of marked idiosyncrasy are not uncommon the effect produced being more profound and prolonged. A large dose should not be given without testing for idiosyncrasy. Barbiturates are divided into two groups:—

(i) *The stable barbiturates.* The stable compounds are slowly decomposed in the tissues and exert a prolonged effect. Such compounds are:—Barbitone B.P., veronal (diethyl barbituric acid) which is insoluble. Dose 5 to 10 grs. Its soluble sodium salt—soluble barbitone B.P., or medinal—dose 5-10 grs.

Phenobarbitone B.P. or luminal or gardenal which is insoluble. Dose ½ to 2 grs. Its sodium salt which is soluble sodium luminal. Dose ½ to 2 grs.

Dial or allobarbitone B.P.C. Dose ½ to 3 gr.

These compounds are slowly destroyed. 90 per cent of barbitone given by the mouth is recovered from the urine. The barbiturates of the second group cannot be recovered from the urine. The effect of first class lasts at least 12 hours and may last for 48 hours. The B.P. dose should not be exceeded. The fatal dose of phenobarbitone is 120 gr but a quarter of that may be fatal in healthy subjects and less in seriously ill patients. Repeated doses may produce toxic effects. The chief symptoms being a rash which is very itchy and papular; oedema of subcutaneous tissues may occur; fever, nausea and mental confusion and even motor paralyses.

Luminal is a very useful drug in epilepsy being superior to bromides. It produces less mental dullness. Dose 1 to 4 gr a day.

(ii) *Unstable or short action barbiturates* which are rapidly decomposed in *the fever* and their action lasts from 4 to 6 hours.

Butylethyl barbituric acid or butobarbital or soneryl. Dose 1½ to 3 gr (0.1 to 0.2 gm).

Pentobarbitone or nembutal. Dose 1½ gr or less (0.2 gm). Other compounds with similar action are hebaral, pernocton, phenodorm, seconal, sodium salts are used in the same dosage when soluble salts are required. They are prepared in cachets to guard against decomposition.

Barbiturates of this class are the best hypnotics for insomnia. They are readily absorbed, leave no sleepy feeling next morning and repeated administration is not followed by toxic symptoms and tolerance is not readily established.

Barbiturates of this class are used as basal narcotics. They are also useful where repeated dosage are required as for convulsive states.

Haxobarbitone B.P. and its soluble salts B.P. Evipan (Cyclonal Hexostab) and pentothal and its sodium salts are short action barbiturates. These are the only two non-volatile narcotics which are used to induce moderately deep surgical anaesthesia without very grave risks of prolonged unconsciousness. These drugs have been used as general anaesthetics by the intravenous route. Pentothal is best suited for operative treatment of wounds. Anaesthesia is induced by 0.5 gm of sodium salt given rapidly into the vein and maintained if necessary by slow administration.

Many proprietary remedies are on the market which contains an analgesic such as amidopyrine and a narcotic, usually a barbiturate.

Bromides. These are not, strictly speaking, hypnotic drugs, but they depress the motor functions, leading to a condition of dullness and apathy which conduces to sleep and is, therefore, particularly useful in nervous insomnia. The effects are generally different from those of the other hypnotics, the bromides produce a mental calm, progressing to sluggishness, lack of attentive power and muscular weakness. All these dispose towards sleep. If the dose is not excessive, perception, observation, and motor functions are not interfered with, but with excessive doses or after prolonged administration, psychic depression and confusion of speech are evident. As hypnotics bromides of sodium, potassium and ammonia are generally used. They are quite effective in doses of 15 to 30 grains, given one to two hours before retiring. In epilepsy, the bromides prove very successful in quite a large percentage of cases. In most cases the attacks become milder and less frequent, and may be suppressed for the time being. Bromides have, however, little action on pain.

Hyoscine. In cases where insomnia is due to acute mania it is used as a powerful narcotic. It is said to increase the action of the heart and circulation without producing any effect on respiration or causing dryness of the throat. In the dose of as little as 1/480 grain it produces its brief mydriatic effect within 18 minutes. It should be avoided in glaucoma.

In all cases where there is a condition of cerebral hyperexcitability, *e.g.,* in chorea, diathermasia (thermal stroke) acute mania, delirium, tremors and post encephalitic Parkinsonism, hyoscine is indicated in doses of 1/50 to 1/200 grain hypodermically.

3. Pain and its Causation

The commonest and the most important symptom for which medical aid is sought is pain. Pain causes intense suffering and a good deal of exhaustion and is a condition which demands instant relief. It is the sacred duty of the medical profession to try to relieve pain by all the remedial means at their disposal. Attempts have been made for a very long time to find drugs which will relieve pain and lessen the suffering of the patient. Opium, the pain-relieving drug *par excellence,* was discovered years ago but time has shown that there are many limitations to its safe administration. The advance of modern organic chemistry has brought within our reach a host of new synthetic analgesic remedies, which though not as potent as opium derivatives are very useful in milder types of pain. Where pain is severe, only opium and its derivatives are effective.

When pain is present, an endeavour should be made to find out its exact cause and nature specially when it is intra-abdominal and intra-cranial origin. In fact, administration of analgesics specially opium and its alkaloids in acute abdominal emergencies is contraindicated until a correct diagnosis has been made; alarming results have been reported where this rule has been overlooked. The exact mechanism of pain, specially abdominal pain, is still a matter of surmise. The different situations where pain is felt or radiates, and the history of the patient, are valuable guides to diagnosis.

Types of pain.

Pain comes from the word 'paena' or punishment. It is described as bodily suffering due to irritation of a sensory nerve, or possibly in rare cases, to changes in the central nervous system. Pain may occur in connection with the rhythmic contractions of uterus during labour or after pains on account of its efforts to expell the clots. Pain with sensation of bearing down may occur in labour or inflammatory affection of female pelvic organs. Lightening pain is the intense shooting pain affecting principally the limbs of patients suffering from locomotor ataxia; girdle pains and sensation of cord tied round the waist is a symptom of organic disease of the spinal cord. Growing pains occur in the joints in rheumatism. Starting pain is caused by spasmodic contraction of the muscles just before the onset of sleep. It occurs in joint disease when the cartilages are ulcerated.

"Sun pain" occurs chiefly in the frontal region early in the morning, specially in cases of inflammation of frontal sinus.

Abdominal pain.

Pain is, with few exceptions the most prominent symptom of an acute abdomen and the most careful and accurate observer may come to an erroneous conclusion. The mechanism of pain is of vital importance in making a diagnosis. Intraperitoneal viscera are insensitive to ordinary pain stimuli. The mesentery on the other hand is extremely sensitive and a mechanical stimulus such as pinching with forceps causes intense pain. Greatest sensitivity is found along the large blood vessels of the mesentery where the nerve trunks are congregated. The greater omentum is insensitive, but the lesser omentum is as sensitive as the mesentery. Pain originating from the mesentery may be severe but is not accurately localisable. The parietal peritoneum of the anterior abdominal wall is very sensitive and even a light touch gives rise to acute pain which can however, be

Sensitivity of organs

localised with a high degree of accuracy. Elsewhere in the body the parietal pleura, the conjunctive and the cornea are the only organs possessing acute sensitivity to pain. The pelvic and posterior parietal peritoneum are also sensitive. The intra-peritoneal viscera though insensitive to the common pain producing stimuli are abundantly supplied with afferent nerves which run without any relays to the posterior root ganglion. Pain in the viscera can be felt only by stimuli of increased tension of the muscular walls as for instance produced by exaggerated peristalsis. Examples of visceral pain are the intestinal colic and the biliary colic.

Headache.

The mode of production of headache cannot be explained easily as the brain substance itself is insensitive to mechanical stimulation. The duramater on the other hand is sensitive and is freely supplied with nerve filaments from the trigeminal; the arachnoid contains no nerves while the piamater does receive twigs from the sympathetic, for its blood vessels. It is now generally agreed that the dural branches of the trigeminal are concerned in the production of headache, as after the extirpation of the Gasserian ganglion the headache rarely occurs on the same side of the head. Headache, like the acute abdominal pain may often be the first symptom of an existing grave disease and needs careful investigation of its cause.

Referred pain.

Referred pain. Much confusion exists in the minds of clinicians on account of the varying significance attached to the term referred pain. Referred pain has been defined as pain projected from a deep-seated point of stimulation to the somatic (or cerebrospinal) sensory nerves on the surface of the body. The exact mode of production and nature of referred pain is still unknown. It was thought at one time to be a feature of visceral disease only but recently it has been shown that similar pain can be set up from appropriate somatic structures. The entrance of an abnormal stream of impulses from an abnormal viscus may alter the state of spinal grey matter (posterior horn) so that it becomes an irritable focus' and normal impulses entering from corresponding segments of the body will give rise to exaggerated or painful reactions in consciousness. Or referred pain should be regarded as nothing other than imperfectly localised pain."

Nature of referred pain.

4. Opium Derivatives and other Analgesic

Opium and morphine.

Opium is the juice of unripe poppy capsules concentrated to a sticky brown mass by evaporation. It is one of the oldest drugs used by man, and the practitioner should be familar with its properties and use. Morphine is the most important of the active principles of opium; there is also meconic acid in it which gives red colour with nitric acid and this is used as a test for the presence of opium in vomits, etc., after poisoning. Morphine acts on the brain and produces a narcotic action, a special action on the perception of pain, a depressing action on the respiratory centre and the cough reflex; it also acts on the sweat glands, pupils and sphincters and inhibits the peristaltic movements of the gut by its local action. All narcotics relieve pain but not till the drug has been given to make the patient almost stuporose; morphine relieves pain in doses which are not stupefying.

Other opium alkaloids.—The average composition of opium is as follows:—Morphine 10 per cent, narcotine 6 per cent, papaverine 1 per cent, codeine 0.5 per cent, thebaine 0.3 per cent. The alkaloids belong to two chemical groups.—*Phenanthrene group* to which belong morphine, codeine and thebaine & *Isoquinoline group* to which belong papaverine and narcotine.

Action of opium alkaloids.

The chief difference in the two is in their action on the visceral centres in the brain. The first group stimulate these centres and are responsible for vomiting and spasm of sphincters while papaverine and narcotine depress these centres. Both groups have the same local effect on the Auerbach's plexus and depress peristalsis; in man this local action is much more marked. Morphine is the only alkaloid which has a powerful pain relieving action. Opium with all its alkaloids and the preparation papaveratum are not smperior to morphine or less likely to excite vomiting. Morphine has not yet been synthesized though many of its derivatives have been prepared. Codeine (methyl morphine) has no pain relieving action and has half the narcotic action of morphine and half the depressant action on the peristalsis; it is less likely to produce vomiting and has no tendency to habit formation. It relieves cough. *Codeine Phosphate* is in the B.P. and its dose is 1/4 to 1 grain.

Artificial derivatives.—A number of alkaloids have been synthesized of which two are important:—

Heroine (diacetyl morphine) is twice as effective in relieving cough as morphine, but its pain relieving action is about the same. Its great disadvantage is that it encourages

habit formation and for this reason its sale is prohibited in U.S.A. It is recognized by the B.P. as *diamorphine hydrochloride*, dose 1/24 to 1/8 gr. It is rarely if ever prescribed.

Apomorphine is another morphine derivative. It has a slight hypnotic action after initial vomiting has passed off. It has a selective action on the vomiting centre and is used as an emetic.

Morphine in pain.

Morphine in relief of pain. Morphine and heroine surpass all other drugs in relieving pain. Amidopyrine and the salicylates possess a definite selective action on the pain centre, but their action is very mild; increase of dose do not increase their analgesic effect; local applications, such as, warmth, local irritants have also a limited effect. It is essential that the practitioner should distinguish between the minor pains capable of being relieved by aspirin, phenacetin, amidopyrine, etc., from severe pain which can only be relieved by morphine.

Morphine in cardiac pain.

The acute pain due to the disease of the heart can only be relieved by morphine. When the pain is due to coronary occlusion and the patient has to be kept at rest, morphine should be given in adequate doses. It should also be given in dyspnoea of heart disease whether accompanied by pain or not. In paroxysmal attacks of dyspnoea due to failnng left ventricle, morphine gives relief on account of its depressant action on the respiratory centre; slowing of the rate and depth of the respiration tend to relieve the added strain which dyspnoea throws on the heart. The relief afforded in these cases is dramatic with a dose of 1/4 gr or less. In early stages of lobar pneumonia the pain is often severe due to pleurites and morphine is indicated. Morphine also relieves irritative cough of tuberculosis of lungs in very small doses, 5 minims of 1 per cent solution of morphine hydrochloride (1/22 gr of morphine) keeping the patient quiet for the whole night.

Morphine in insomnia.

Morphine in insomnia. 1/4 grain dose may send the patient to sleep but there may be preliminary excitement accompanied by nausea lasting for some hours. Although morphine will certainly give sleep to most of these who are in pain, it is an uncertain hypnotic in painless conditions.

Duration of morphine action.

Duration of action of morphine. Morphine is excreted mostly by the kidney, the excretion by the intestinal route being of little significance in man. After a dose of 1/4 grain of morphine, pain will be relieved for eight hours and sufficient quantities will remain in the blood for another twelve hours to produce a feeling of sleepiness, nausea and vomiting. After a very large dose, *e.g.*, one grain, the subject remains in a state of coma for about six hours; after that there is no risk of death by failure of respiration though the subject may remain unconscious for another twelve hours and very sleepy for a day or two longer.

The official B.P. dose of morphine is 1/8 to 1/3 gr. Such doses may be fatal in the very young or very old; on the other hand 1/3 grain may not relieve severe pain in heavy patients. A safe initial dose should, therefore, be given at first and if necessary more should be given till desired effect is produced. Repeated dosage is often not necessary. In an average sized adult 1/6 to 1/3 grain will relieve severe pain. In vigorous adults free from respiratory embarassment over one grain may have to be given. In very young and very old it should be given with the greatest of caution.

The following table from Clark (1933) gives the expert report of the League of Nations (1931) about certain morphine derivatives.

Drug	Mean therapeutic dose in grammes	Relative toxicity	Relative power of producing addiction	Relative action as analgesic	Relative depression of respiration and cough	Relative depression of gut movement
Morphine	0.02 gm	1	1	1	1	1
Codeine	0·04 „	½	very slight	very slight	¼ to ½	½
Heroin	0·005 „	5—10	2—4	2	2	very slight
Dicodide	1·005 „	...	½	?	1	?
Dilaudide	9.003 „	...	½	1	¾	very slight

Pethidine.

Pethidine. Pethidine (1-methyl-4-phenylpiperidine) also known as 'Dolantin' or 'Demerol' is a comparatively newer addition to the list of analgesic drugs. Chemically it is related to drugs highly reputed as pain relieving agents like morphine and tropic acid series of antispasmodics.

Pharmacological investigations show that it is a powerful antispasmodic and does not affect the pupil or the pulse rate. It is a strong analgesic, raising the threshold of pain. Its toxicity is relatively low; it depresses, respiration and lowers blood pressure only with large doses. It thus appears to be a very suitable analgesic but carefully controlled clinical trials are still needed before coming to definite conclusions.

Pethidine has been given some trials in obstetric cases, renal and biliary colics, in pain due to injury or operation and in neuralgias. The results of these trials are very encouraging. In obsetetric cases particularly it has proved successful in relieving pain without any adverse effect on the mother or child.

An ideal analgesic should be rapid, dependable, safe, having no adverse side effects and without tendency to habit formation. Pethidine can be administered by mouth as well as parenterally and within 5 to 15 minutes of intravenous administration analgesic effect lasting for 3 hours ensues. Undesirable side effects such as nausea, vomiting or vasomotor disturbances may occur but are mild and of short duration. There is no cumulative effect. However the withdrawal syndrome although mild does follow on stopping pethidine after administration for sometime and the drug carries certain risk of addiction. In Germany indiscriminate use of this group of drugs has already been stopped.

Aromatic analgesics.

A large number of coal tar derivatives are commonly used to lower temperature and to relieve headache and pain. They have analgesic effect against neuralgic and muscular pains, headache, migraine, cold, etc., but are often ineffective if the pain is due to some injury.

Aspirin.

Aspirin (acetyl salicylic acid), is the most popular remedy for headache in doses of 5 to 15 grains. Nothing very definite is known as regards the etiology of headache. A rise in the pressure of the cerebro-spinal fluid is a frequent cause of headache while the spasmodic contraction of the cerebral vessel may probably be responsible for migraine. The manner in which these drugs act is uncertain, and Barbour believes that acetyl salicylic acid causes a transference of water from tissue into blood and thereby relieves headache. The depressant effect of the drug on the heart precludes its continuous use. Phenazone or antipyrin and phenacetin were formerly used as analgesic to relieve headache, migraine, facial neuralgia, etc., but they are now less frequently used. Pyramidon or amidopyrin acts similarly to phenazone and is effective in doses of 5 to 8 grains. It is an efficient analgesic and its toxicity is very low. Veramon is a combination of amidopyrin with diethyl barbiturate and is said in many cases to relieve pain as well as morphine; Allonal, a combination of allyl isopropyl barbituric acid with amidopyrin has both analgesic and hypnotic effect. Cibalgin, a compound of amidopyrin with dial, is given in doses of 4 to 16 grains. Compral, a new analgesic, is a combination of pyramidon with trichlorethyl urethane for use in pain generally; one to two tablets of 7½ grains each will be useful.

Veramon.

CHAPTER VII

PYREXIA

REGULATION OF BODY TEMPERATURE—PYREXIA AND ITS TREATMENT—ANTIPYRETIC AND ANALGESIC DRUGS—TREATMENT OF FEVER—EFFECTS OF HEAT (HEAT STROKE): HYPERPYREXIA, HEAT EXHAUSTION, HEAT CRAMPS, PRICKLY HEAT.

1. Regulation of Body Temperature

The normal body temperature.

The human body is adjusted to work best at a certain temperature, which is called the normal. Any variations from it are detrimental to the proper working of the organism. Provision is therefore made in the body to regulate the temperature and keep it at the required level. In the cold-blooded or poikilothermic animals, the temperature depends on the surroundings. It rises in hot weather and falls in the cold and the life of the animal varies accordingly. In the warm-blooded or homo-thermic animals, the temperature is maintained at a constant level independently of their surroundings. Transitional forms between these two types occur such as in hibernating animals. The mean body temperature maintained by warm-blooded animals differs in different animals, *c.g.*, among the birds it usually lies between 40°C. (104°F.) to 43°C. (107.6°F.) or more. In most mammals, the mean body temperature is lower than birds' but somewhat higher than that of man.

Variations.

Normal variations also occur in the body temperature in human beings. The normal temperature in man is usually taken to be 37°C. or 98.4°F. but it is well known that the temperature differs in different parts of the body and also at different times of the day. The rectal temperature is nearly 0.6°C. or 1.0°F. higher than the mouth temperature; the temperature in the axilla is 0.6°C. or 1.0°F. lower than the mouth temperature. As regards the diurnal variations, the temperature rises during the day and attains its maximum late in the afternoon (37.5°C. at 6 p.m.); it falls during the night and reaches its minimum in the early hours of the morning (36.2°C. at 4 a.m.). The difference in the maximal and minimal temperature usually amounts to somewhat over 1.0°C. or 1.7°F. The cause of these variations is not clearly understood, but is attributed by some to muscular activities during the day and rest during the night. Food has also something to do with it, as diurnal variations of temperature are not so marked during starvation. The temperature also varies at different periods of life. The temperature in the infant is higher than in later life. The body temperature rises several degree after exercise (1 to 4°F. per rectum, but oral temperature may remain the same). Temperature after exercise takes 30 to 60 minutes to return to normal.

Regulation of body temperature.

Apart from the small diurnal variations, the temperature of the body is maintained at a remarkably constant level by the physiological processes in the body. This is accomplished by a fine adjustment of heat production and heat loss. Any change in the environment which involves a marked increase or decrease of temperature quickly brings the heat-regulating mechanism into action. The temperature of a normal person rises when muscular work is undertaken. A brisk walk for two hours may raise the rectal temperature of a healthy person from 37.0°C. to 38.4°C. (101°F.). In certain diseases such as tuberculosis the diurnal variations as well as the rise of temperature produced by exercise is increased. The brain can only function normally within a narrow range of temperature. A fall of 3°C. below normal produces narcosis, whilst a rise of more than 4°C. above normal may produce delirium.

Heat production is governed by: (1) The motor nerve centres and motor nerves to muscles and glands. (2) The quantity and quality of the food consumed. The heat generated depends on the amount of food oxidised and the activity of glands and muscles. Thus a starving animal shows a lower temperature than one fed normally. Although muscular activity plays a very important part in the production of heat, it has been shown that even if the voluntary muscles are paralysed by administration of curare, a high temperature can still be maintained in febrile animals. The production of heat in these cases must apparently be due to the activity of glands and involuntary muscles. Metabolism and the body activity of the voluntary and involuntary muscles and of the glands are, therefore, important factors in the production of heat.

Basal metabolic rate.

The heat produced depends upon the basal body metabolism and upon excesses above this resulting from muscular activity and the ingestion of food. The basal metabolic rate (B. M. R.) of an individual means the production of heat with the body at complete

muscular repose and fasting. The rate is subject to marked physiological fluctuation. According to Benedict the average basal metabolism of a normal adult is about 25 calories per kilo. of body weight per day. The B. M. R. is usually calculated in calories per sq. meter of body surface. If height and weight of an individual are known, his body surface can be calculated from tables. The normal B. M. R. is 40 calories per sq. meter of body surface per hour. Lighter individuals though showing a smaller total metabolism than heavier ones, usually have a greater metabolism per unit of body weight. The heat production is increased by exercise, by food and exposure to cold. A normal man of sedentary habit produce about 3,000 calories a day, while a manual labourer produces as much as 6,000 calories per day.

Heat loss.

The heat is lost mainly through, (*i*) radiation and conduction from the skin, (*ii*) evaporation of water from the skin and respiratory tract. Heat is lost also through the excretions, in the process of warming the inhaled air and the ingested food taken at a lower temperature. In temperate climates, approximately 75 per cent of heat is lost by conduction and radiation if a man is doing some physical work. During rest, heat loss through evaporation comes more prominently into play. If the heat loss is suddenly increased by the application of cold to the surface, there will be a reaction on the part of the body resulting in constriction of the peripheral blood vessels and shivering. The constriction of blood vessels is brought about by the vasomotor centre in the medulla which ensures that the blood supplied to the vital organs is maintained at a proper temperature. The constriction will lessen the amount of blood circulating through the skin vessels and thus reduce the heat loss through the skin. Shivering will increase heat production and by diminishing heat loss and increased heat production the effects of sudden cooling will be counteracted. Increase in the surrounding temperature will, on the other hand, involve the reverse kind of reaction by the same heat-regulating mechanism. There will be a dilatation of the skin vessels and increased perspiration. Both these factors will prevent the body temperature from rising above the normal, by increasing the heat loss from the body. The temperature is thus kept constant by changes in the production and dissipation of heat.

Heat loss from the body usually amounts to 70 per cent from radiation, convection and conduction, 14.5 per cent from evaporation of sweat, 8.0 per cent from vapourization of water from the lungs and about 7.0 per cent from other sources.

The delicate adjustment of heat production and heat elimination is under the control of the central nervous system. The principal centres concerned in heat regulation lie near the basal ganglia and control both the production and the dissipation of heat. They in their turn are influenced by afferent stimuli from the skin and mucous membranes as well as by the temperature of the blood which circulates through them, and they work in close co-operation with the vasomotor respiratory and the sweat centres.

Regulation against over-heating.

If the amount of heat generated is greater than that eliminated, a rise of body temperature is the result. Production of heat is increased after food and especially after protein food, but this increase is never great and is promptly eliminated. Overeating especially by consumption of meats may increase discomfort during hot weather by throwing an extra burden upon heat elimination, but this rarely causes any appreciable rise. Muscular activity, on the other hand, is one of the important causes of over-heating. It has been seen that during moderate exercise the total metabolism of the body may be increased 2 to 3 times and after violent exercise 5 to 8 times the normal resting metabolism. With the increase in metabolism, the temperature of the body rises and it is quite common to see an increase of 2° to 3°F. in the rectal temperature. An equilibrium is, however, maintained by increased dissipation, by radiation, conduction, evaporation from the surface of the body and by deep breathing. When this elimination is defective, a serious rise of temperature may occur and heat stroke may result. This phenomenon is very well illustrated in the case of certain animals like the dog in which perspiration is almost absent. If evaporation from the tongue is prevented in such an animal, by making him breathe through a tracheotomy cannula and the animal is made to take exercise, the temperature rises so high, following a brief period of muscular exercise, that the animal may die of heat stroke. In man, in certain diseases of the skin, *e.g.*, icthyosis, the power of dissipation of heat through the skin is interfered with and in these patients muscular exercise may produce an unusual rise of temperature. If the external temperature is higher than that of the body, the heat loss is not carried out by conduction but by evaporation of sweat, which is secreted to a greater extent on account of an increased flow of blood in the cutaneous vessels. In man this is the chief means of regulation of temperature if the surrounding temperature is high. The nerve centres are guided in this regulation, (1) by

the temperature of the blood coming to them and (2) by reflexes from the skin. In man reflexes from the skin play a very important part. The rate of evaporation depends on the humidity of air. Movements of the air assist in evaporation from the skin and excessive heat is borne better when the air is in motion. In tropical climates, where the external temperature is high, the increased dissipation keeps the temperature down, at the same time the individual feels relaxed, restricts his exercise and food and thus helps in diminishing heat production.

Regulation against over-cooling.

Man can tolerate a much greater range of low temperature, protection against heat loss being assisted by a thick layer of fat under the skin which is a bad conductor of heat. In animals, fur and other covering, and in man clothes prevent dissipation. Besides this, two other factors are important: (1) Physical regulation, *i.e.,* by constriction of cutaneous vessels so that less blood flows through the periphery. Even exposure to moderate cold reduces the cutaneous supply of blood of the arm to one-half or less of what it is at a comfortable temperature. (2) Chemical regulation, *i.e.,* by increased metabolism in the muscles as shown by shivering. This only comes into force when the physical regulation does not suffice. After division of the dorsal cord the metabolism fails to show the characteristic change when the animal is exposed to cold, because of the paralysis of muscles. Extra heat is also obtained by ingestion of food. As in the case of over-heating, the mechanism is under the control of nerve centres which are influenced by sensory impulses from the skin. If the skin is suddenly cooled a reflex constriction of the cutaneous vessels of the body occurs immediately. A cold bath often causes a rise of temperature by increased muscular activity by shivering and constriction of skin vessels.

2. Pyrexia and its Treatment

Conception of fever.

Fever is derived from Latin word *febris* meaning to boil. It is a complex phenomenon, the main feature of which is a rise of the body temperature associated with disturbance of metabolism, special senses, pulse, respiration, etc. The rise in temperature in fever is due to a defect in the heat regulating mechanism, which is not clearly understood. Physiological fever, we have already seen, is produced by immersion in a warm bath at 40°C. and is not accompanied by any of the other phenomena of fever above described. Entrance of living or dead bacteria or their products in the blood or protozoal organisms produces fever. No constant relation exists between the severity of infection and the height of fever. The cause of marked fluctuations in temperature during infections is not understood. In some it is merely an exaggeration of nomral diurnal variations. Other conditions giving rise to fever are injection of foreign proteins, destruction of cells in the body in the absence of bacteria (*e.g., extravasated blood*) and a number of chemical poisons. The injection of certain salts, sugar, sulphur, atropine, caffeine, cocaine, strychnine, picrotoxin, tetrahydro-beta-naphthyl-amine, epinephrin, and many other substances will increase the temperature when given in large doses. Puncture of the brain in the region of the corpus striatum produces fever which is associated with constriction of the peripheral blood vessels and an increased production of heat within the body, mainly from combustion of carbohydrates derived from hepatic glycogen. In a general way the rise resembles that which occurs during infective processes; essential and undoubted differences have not been established between the two. The probable explanation is that the rise of temperature in infectious disease is due to stimulation of the heat regulation centres by some products of infection. Clinically, rise of temperature occurs after cerebral hæmorrhage, especially into the ventricles, due to mechanical or chemical irritation of the basal portions of the brain. Injection of water also produces a rise of temperature, the cause of the so-called water-fever probably being the presence of minute traces of organic matter (pyrogens) in the water used for injection. Excessive loss of water may lead to elevation of body temperature.

Heat pyrexia.

With sweating there is a considerable loss of salt. It has been shown that the ratio of potassium to sodium in the sweat is greater than in the plasma and after an exposure to tropical conditions a definite fall in the potassium concentration of the plasma occurs.

The dangers of excessively warm conditions are twofold. The first is the danger of collapse from diminution of the distension of the circulation resulting partly from a dilatation of the circulatory bed and partly from a loss of fluid into the intercellular spaces and through the sweat. The second danger is that an excessive loss of K changing the ratio of Na: K may be the cause of hyperpyrexia as a result of which the temperature continues to be maintained at a dangerously high level or to mount still higher even after removal of initial cause. There are, of course, conditions so severe that no device will prevent a fatal rise in temperature, but below this impossible level the danger of

failure of the circulation may be avoided by an adequate supply of sodium chloride and water, and danger of hyperpyrexia by an adequate supply or reserve of potassium. The advantage of the first is confirmed by a wide range of practical experience; the second is, it should be emphasized, speculative.

Heat regulator in fever.

Fever may be continuous, remittent and intermittent. The temperature may fall by lysis or crisis. The febrile temperature is primarily due not to an increase in the heat production or to an absolute inefficiency in heat dissipation but to a lack of adjustment between the two. The heat regulation in fever behaves as if the regulatory centres were set to maintain the body temperature at a new level, which is maintained in the same way as normal temperature. In fever the heat regulation is less perfectly adjusted than in health as it is much more difficult to reduce the temperature of a healthy man than a fevered individual either with cold baths or with antipyretic drugs. The extra heat produced within the body is also less perfectly eliminated.

Metabolism in fever.

Metabolism is accelerated in fever as warmed tissues metabolise more material than cooled tissues, but in addition to this there is probably a toxic element that causes tissue destruction, and the rapidity of protein consumption varies in different infections. This increased metabolism rarely exceeds 40 or 50 per cent over normal. No strict parallelism exists between the rate of protein decomposition and elevation of the temperature. The amount of protein lost to the body may be considerable, being as much as 200 to 500 gm. of muscle tissue per day. In surra more than one-fifth of the proteins of the body are lost in the course of the disease. Immediately following the cessation of fever there is a marked increase in the nitrogen excretion. The nitrogenous equilibrium can be maintained in fevers and the patient's strength conserved by giving adequate diet, but unfortunately in many fevers the toxins produce loss of appetite which prevents intake of food and a decrease in absorption may occur. The partial starvation thus produced increases protein metabolism; besides high temperature it produces further rapid wasting of tissues. To supply this need administration of glucose and other similar foods is necessary.

Acidosis in fever.

Acidosis also occurs in fever but its occurrence is probably not as common as is supposed. Ketonuria which often occurs in febrile conditions is an indication of disturbance in fat metabolism, but is not considered as evidence of acidosis.

It is well known that excretion of chlorides in fever is decreased. This is certainly true of pneumonia and other infectious diseases. The retention of salt is associated with simultaneous retention of water, especially in long continued fever such as typhoid; the tissues, therefore, become relatively rich in fluids and poor in solids. A normal individual will excrete up to three litres of water which may be introduced into his body; the fever patients do not do this and retain a considerable amount. The extra water is, however, stored mainly in the tissues and less in the blood and the same is the case with the chlorides. The state of affairs is, therefore, different from what occurs in nephritis where the concentration of the salt is high in the blood. It has been shown that antipyretic drugs bring about a dilution of the blood, which favours dissipation of heat by radiation.

Effects of fever.

Heat above normal body temperature, especially if continued for a long time, causes degenerative changes in the tissues. The cells undergo cloudy swelling. The proteins of the muscles coagulate at 104°F. (40°C.) and in nearly all tissues globulin coagulates at 45°C.; mammalian muscle passes into rigor between 45° to 50°C. Coagulation takes place at a lower temperature when continued for a long time. Even if coagulation is not visible a change analogous to it may cause death of the cells. It is possible that heat and toxin may act in a synergistic manner to disturb the functions of the cells.

Cloudy swelling.

Pulse rate.

The heat also acts directly on the heart muscle and stimulation of the nerve centres controlling the heart produces slowing or acceleration. When temperature is the only factor, a rise of temperature, *e.g.*, 97.8° to 104°F. (37° to 40°C.) will cause an increase of pulse rate. In addition to this, toxins play a part, *e.g.*, in typhoid fever the pulse is relatively slow, being about 70 to 80 beats per minute with a temperature of 103° to 104°F. In scarlet fever the pulse is surprisingly rapid.

As regards the central nervous system, the toxins and other pyretics are said to produce a stimulation of the regulatory mechanism. A number of drugs, *e.g.*, cocaine, atropine, caffeine, epinephrine, which are known to stimulate the central nervous system, cause a rise of temperature, while those which depress the nervous system, *e.g.*, alcohol, chloral, morphine, cause a fall of temperature. The mechanism of production of fever seems to be a stimulation of the sympathetic system to which the heat regulating mechanism probably belongs. The other convulsant poisons such as santonin, picrotoxin and phenol,

which stimulate the para-sympathetic system, and cause myosis, slow pulse and psychic depression, do not increase the temperature.

The significance of fever.

The practical question involved in the treatment of infectious diseases is to what extent should one atempt to reduce the temperature. It was thought that the febrile rise of temperature was on the whole a favourable manifestation and helped the organism in overcoming infection. Clinical experience shows that when bacterial infections are unaccompanied by fever they run a very unfavourable course. Leprosy, tuberculosis and parasitic diseases not marked by febrile reactions are usually difficult to cure and do not confer immunity. Treatment of neurosyphilis by artificial infection with malaria probably chiefly acts by increase of temperature. Collapse may occur in certain conditions if the stimulus of high temperature is removed. It has been shown that with most bacteria the optimum temperature for development is confined within very narrow limits, which are exceeded by the temperature of fever. The tendency of fever may, therefore, be useful, but unfortunately the cells of the organism are not adjusted to work under high temperature and it may be more detrimental to them than to the bacteria. It has been advocated by some that fever is the essential manifestation of infection, that it is dangerous in itself, and should be vigorously combated. The question still remains unsettled, as patients with a high temperature are more sick than those with a low temperature, but this is not due to the temperature but because such patients are suffering from more severe infections. Besides this, in the most unfavourable of all infections the temperature may be low. From a comparison of clinical cases it is, therefore, not possible to judge the favourable and unfavourable effects of temperature. The antipyretic measures also do not give any clear answer, because not only do they reduce the temperature, but they have various effects on other parts of the body, especially the brain and the circulation.

Effects of Pyrexia on course of Infection.

Many experimental studies have been carried out in this connection. Rabbits have been kept with a body temperature of 41°C. (105.8°F.) and over for weeks at a time without any serious damage. The degeneration of internal organs observed in infectious fever is probably not due to prolonged overheating of the body. Serious effects are only produced when the temperature is raised to a height not usually encountered in fevers. The damage appears to be done more by the infection. The question is, does the rise of temperature exert a favourable or an unfavourable effect upon the course of infection? The high temperature may have a direct effect on the infective organism or it may help the host in developing protective mechanisms. The temperatures encountered in certain fevers are sufficiently high to inhibit the growth of certain bacteria. Pneumococcus and gonococcus do not grow well at a temperature of 104°F. (40°C.) or over. Moderately high temperature, even though maintained for a long time, is not in itself a dangerous manifestation. In artificial infections in animals an increased temperature has a favourable effect on the course of the infection and increases the speed with which the protective anti-bodies are formed. In the treatment of fevers, therefore, one should not try to bring down a high temperature too vigorously unless it is above 105.8°F. (41°C.). In such cases the reduction of temperature is comparative; but in ordinary febrile temperatures there is no need for active interference. The value of antipyretic measures in such cases is to be judged, not solely from their effect in reducing the temperature, but also from their effect on the general condition of the patients and especially on their nervous system and circulation.

Antipyretic measures.

The temperature of the body can be lowered by factors which decrease the production of heat or increase its loss. As the temperature tends to fall below normal, compensatory reactions are at once set up to keep the temperature normal. It is not found possible to depress the temperature without effecting profound changes. On the other hand, it is relatively easy to lower an abnormally high temperature because it coincides with the natural tendency to return to the normal or physiological state. Antipyretics, therefore, have no effect on normal temperature but readily act when pyrexia is present. Antipyretic measures can be divided in three main groups:—

(1) Those that abstract heat, *e.g.*, application of cold in form of baths, sponging, packs. This subject has been discussed under physiotherapy.

(2) Those that increase the dissipation of heat by dilating the cutaneous vessels and by restoring the water content of the blood. Their action is mainly central. These are antipyretics of the coal tar group, *e.g.*, acetanilide, phenazone (antipyrin), phenacetin.

(3) The cinchona alkaloids, especially quinine, which is said to diminish heat production. Salicylates, aconite and veratrum probably diminish heat production by slowing the circulation.

3. Antipyretic and Analgesic Drugs

Antipyretic drugs.

High temperature in the old days was chiefly combated by baths and giving such vegetable alkaloids as aconite and quinine. It was discovered as early as 1875 that salicylic acid and related bodies produce a fall of temperature in fever. Most of the modern antipyretics were discovered in an attempt to synthesise quinine. A large number of bodies were introduced, but those of importance are acetanilide, phenazone, phenacetin and amidopyrin or pyramidon. These are all benzol derivatives and prepared from aniline. Phenol, guaiacol, salicylates and many other compounds also lower febrile temperature, but they are much inferior. All these drugs have the same action. The antipyretic drugs are divided into three groups:—

(1) The essentially antipyretic drugs are, (*a*) acetanilide and acetophenetidin or phenacetin and (*b*) antipyrin and amidopyrin also known as pyramidon; they are also analgesic. Like alkaloids these are precipitated by tannic acid, alkalies and other alkaloidal precipitants.

(2) The anti-rheumatic group consists of salicylic acid and its derivatives such as acetylsalicylic acid (aspirin), etc. Cinchophen or atophan also comes under this group.

(3) The antimalarial antipyretics, *i.e.*, the cinchona alkaloids.

Phenacetin.

Acetophenetidin or phenacetin. Phenacetin is a white crystalline powder. The dose is 5 to 10 grains (0.3 to 0.6 gm.). It is much less toxic than acetanilide. It produces a feeling of drowsiness and, therefore, acts as a mild hypnotic; it has a much more powerful action on neuralgic pains than any of the other antipyretics. It is an ideal antipyretic in that it produces a gradual but persistent fall of temperature and does not produce collapse. It does not produce cyanosis and has less tendency to cause skin eruptions and profuse sweating. The antipyretic action is thought to be due to para-amino-phenol, and varies directly with the rapidity with which this substance is formed in the body.

Antipyrin.

Antipyrin or phenazone. It is derived from phenyl hydrazone; the dose is 5 to 7 grains or more (0.3 to 0.5 gm.) and its action is very similar to acetanilide.

Amidopyrin.

Amidopyrin or pyramidon is a derivative of antipyrine. It is given in doses of 5 grains (0.3 gm.).

These drugs have no effect on the normal temperature. Antipyrine is more analgesic in neuralgic pains and neuritis originating from the spinal cord than acetanilide, and is, therefore, largely used in the shooting pains of tabes which it may not always relieve. Phenacetin is used more for headaches. Phenazone is slightly less toxic than acetanilide and little more than phenacetin. A large number of combinations of antipyrine have been put on the market, *e.g.*, salipyrin is a combination with salicylic acid. Antipyrine affects the circulation and the kidneys slightly and can be prescribed in heart disease and nephritis. It is not very irritating to the stomach. It has been used in tuberculosis, typhoid fever, erysipelas and pneumonia but it should be given with great caution.

Cryogenin.

Cryogenin occurs in crystalline masses and is slightly soluble in water. It has been used as an antipyretic to control high temperature, and this is found valuable in the treatment of phthisis and lingering pyrexia that sometimes follows the acute stages of an infection. The dose is 3 to 24 grains, but it should not generally exceed 10 to 15 grains daily.

Salicylates.

Salicylates. These compounds lower the febrile temperature promptly. The mode of reduction is very much like that of the members of the coal tar group, *i.e.*, increased loss of heat by dilatation of cutaneous vessels and increased perspiration. In healthy individuals this is compensated for by increased heat production so that the normal temperature remains unaffected. Salicylates may easily take the place of other antipyretics and are probably safer; but may produce unpleasant side effects. Small doses of acetylsalicylic acid or aspirin (5 to 10 grains) are commonly used in mild fevers and not only reduce temperature but also remove unpleasant symptoms such as headache. Novaspirin (methylene-citrilsalicylic) has no advantage.

In 1937 the citizens of the United States consumed 5,143,672 lb. of aspirin. This works out at 48 five-grain tablets per head of population. This shows the popularity of this drug. After taking aspirin patients often complain of heart-burn and epigastric discomfort presumably because of liberation of a small quantity of salicylic acid in the stomach by the gastric acid. Aspirin lying on the mucous membrane of the stomach may produce irritation resulting in local hyperaemia and even submucous haemorrhage. If taken after

food or with milk, it probably has no deleterious effect. Calcium aspirin (which is much more soluble than ordinary aspirin) causes only slight gastric irritation, and is recommended.

Various constitutional upsets have also been attributed to aspirin from time to time. Aspirin hypersensitiveness is the most common form of drug allergy; and in sensitive subjects *e.g.*, asthmatics with nasal polyps, and people who have a family history of allergy, special caution is indicated. The potential dangers of almost any drug are such that we should not be unduly impressed by rare untoward reactions, and it is not surprising that an allergist should be emphatic about the ill effects of some drugs that the general practitioner regards as eminently safe. Dangerous reactions may occur in sensitive persons but these occur rarely.

Cinchophen and neocinchophen.

Cinchophen or atophan is phenylcinchoninic acid. It has a biting bitterish taste and is irritating to the stomach. It has a pronounced action on the liver cells and increases the quantity of bile. Its absorption from the intestine is irregular and uncertain, and some can be recovered from the urine. Cinchophen is an antipyretic, but its chief action is an analgesic in rheumatism and as a mobilizer of uric acid. The dose is 5 to 15 grains (0.3 to 1.0 gm.).

Neo-cinchophen is an ethyl ester. It is also called novatophan and tolysin. It is tasteless and does not irritate the stomach. It has also a cholagogue action and has less destructive effect on the liver cells than cinchophen. The dose is 8 to 15 grains.

Both these compounds are chiefly used in rheumatism and gout.

Quinine.

The antipyretic action of quinine has been dealt with in another section. In malaria, quinine reduces the temperature because of its effect on the malarial parasites, but in addition it has a general antipyretic effect in fevers other than malaria. Large doses of quinine are said to depress the metabolism by 10 per cent; the heat loss appears to be but little altered. Quinine has also a sedative action on the centres though it is not so marked as with other antipyretics. Quinine, however, conserves energy probably by decreasing metabolism instead of increasing the heat loss. In such diseases as influenza when the depressant action of the aniline drug is undesirable, quinine is very useful. The antipyretic dose is 5 to 15 grains.

Antipyretic action of analgesic antipyretics.

In normal persons even large doses of these drugs produce no effect. In fever a fall is produced beginning within 2 hours and lasting for 2 to 4 hours. The fall is accompanied by profuse perspiration due to dilatation of cutaneous vessels and increased heat loss owing to direct action of the drug on the heat regulating centres. The effect of direct application of these drugs to the region of the heat centres was studied: both the antipyretics and narcotics produced a fall. Central nerve stimulants such as caffeine produced a rise. The mechanism of rise of temperature is a stimulation of these centres and the antipyretics, therefore, act by depressing them.

Toxic effects.

Some individuals show an idiosyncracy to these drugs, cyanosis and collapse being produced with ordinary therapeutic doses. Papular and erythematous skin rashes may follow phenazone and acetanilide, antipyrin produces a scarlatiniform rash with œdema of the face and fever. It may also produce urticaria, or a vesicular, bullous or eczematous eruption. A large number of headache powders contain acetanilide and deaths have occurred from their use, the chief symptoms being cyanosis, nausea, persistent vomiting, weak and irregular pulse, dyspnœa, cold sweat, coma and collapse. Deaths have occurred with 5 grains (0.3 gm.) but recovery is reported after 120 grains (7.5 gm.). Severe symptoms often follow from 0.2 to 1.0 gm. doses. Antipyrin may cause burning and swelling of the whole alimentary tract, with nausea, vomiting, diarrhœa, skin rashes, mental dullness, tremors, cerebral convulsions, coma and death from failure of respiration. In the case of amidopyrin, excitement, increased reflexes, and a measles-like eruption have been noticed. Frequent use of the drug may produce agranulocytosis.

Acetanilide and phenacetin produce acute and chronic poisoning; in one form collapse and cardiac failure being prominent features and in the other cyanosis accompanied by anæmia, emaciation and weakness. There is dyspnœa and the heart is rapid and weak. The cyanosis is said to be due to the formation of methæmoglobin, but it is probably produced by para-amidophenol or some other aniline derivative which has been found in the blood and urine; sulphæmoglobinæmia is said to be present. Methæmoglobin has, however, been seen spectroscopically. Cyanosis may persist for weeks after the drugs are stopped. Caffeine is often combined with these drugs to prevent depression, but it is said to increase the toxicity of acetanilide. Sodium bicarbonate is believed to lower their toxicity.

Treatment consists in washing out the stomach with alkaline solution, maintenance of body temperature and combating the collapse with atropine, caffeine, strychnine, digitalis, etc. Chronic poisoning is met with, the symptoms being cyanosis, anæmia, disturbance of digestion, headache, dyspnœa on exertion, weak pulse, extreme muscular weakness.

Habit formation.

Habit with these drugs is formed by nervous patients suffering from headache, neuralgia, etc. The habitues show mental depression, digestive disturbances, anæmia and general weakness. The habit is not vicious like the morphine habit but it may be difficult to break.

4. Treatment of Fever

From the foregoing discussion it is obvious that pyrexia alone does not constitute the sole indication for treatment. The toxic state of the patient is a more important factor. When it is desired to reduce temperature it is advisable to control it by use of cold sponging or bathing. Antipyretic drugs should be resorted to as little as possible. The objection to these is that they depress the patient and decrease his power of resisting disease.

Fluids.

In all fevers, especially when they are prolonged, water should be freely given whether the patient asks for it or not. Not less than two quarts should be given to an adult daily. It is not only essential to flush the kidneys and remove toxins circulating in the blood, but it is essential for the proper functioning of the body. The turnover of the fluid during digestion has been estimated at five quarts per day, of which no less than one quart is bile, the fluid being excreted and reabsorbed. Considerable quantities of water are lost in the urine and respiration and a certain amount in normal stools. In heat elimination, an important factor is the distribution of water in the body. By dehydrating a dog a high temperature may be produced. The fever is caused by blood concentration and checked by blood dilution. In influenza, typhoid, pneumonia, salt fever, inanition fever and other fevers, the blood has been found to be concentrated, the reason being a special affinity of the tissue colloids for water. When the volume of the blood is diminished, the viscosity is increased and the surface flow of the blood decreases. The result is diminished sweating and consequent diminished heat elimination and fever. The dilution of the blood by increased ingestion of fluids remedies these defects, increases heat elimination and lowers temperature. Antipyretic drugs, such as sodium salicylate, aspirin and cinchophen are said to act in fever by lessening the blood concentration and thus increasing heat elimination. In non-febrile patients, in whom there is no dehydration, there is no fall of temperature in equivalent doses.

Antipyretic drugs.

Analgesics in the treatment of fever.

The chief use of antipyretic measures is reduction of temperature in febrile conditions, but antipyretic drugs have also a sedative action on the nervous system. When the temperature is high enough to produce nervousness, headache and discomfort, antipyretics by reducing the temperature, quieting the nervous system and relieving pain, promote well-being and aid in recovery. Rapid reduction of temperature is, of course, essential in case of sunstroke, for if this is not done the temperature rises steadily till the patient dies. Such cases can only be treated by cold sponging or cold baths. There is general agreement that antipyretic drugs are of little value in the treatment of severe fever, when the temperature is retained at a high level for many weeks. Such cases are benefited by cold sponging or cold baths. Even if the bath may not produce a prolonged lowering of the temperature, the short fall gives the organs of the body rest and the results are very satisfactory. The chief use of antipyretic drugs is in mild cases of fever and in these they generally make the patients more comfortable and often assist in producing sleep, though they may not

influence the course of the disease. They neither strike at the cause of the fever nor at any symptoms other than those resulting from hyperpyrexia. They make the type of disease neither less severe nor shorter. For instance, in malaria, antipyretics may prevent the development of the paroxysm but they do not attack the cause of the disease. They are merely a symptomatic and not a specific mode of treatment and symptomatic treatment should always be carried out with care, as more harm than good may be done. When it is not possible to attack the cause of the disease it is advisable to remove the objectionable symptoms. This they do by their sedative action on the pathologically stimulated centres and on the sensory part of the cerebral cortex. Schmidberg gave them the name of "fever narcotics" and this is a very suitable name, as now-a-days they are seldom employed to combat hyperpyrexia, but rather in hope that the patient will be benefited by their sedative action on the symptoms of fever due to hyper-excitability of the brain centres. They decrease pain in the limbs, headache, delirium, and restlessness, clear confusion of mind, and induce sleep. The effects of these drugs vary with the individuals, some people can take large quantities without effect, while others are affected by small quantities.

When antipyretic drugs are used, it is better to give a small dose before resorting to a large dose. Special care is taken when these are given in diseases where the temperature falls by crisis as a dose before the crisis may produce dangerous collapse by exaggerating the physiological fall.

In cases of pain and discomfort without fever, such as headache, migraine, neuralgia, muscular rheumatism, pains of locomotor ataxia, dysmenorrhœa, and in various nervous conditions, in chorea, whooping cough, diabetes insipidus, antipyretic drugs especially acetyl salcylic acid (aspirin) generally give relief. Pyramidon is said to be a specific for measles. The drugs are usually given in the form of capsules or tablets for internal use and are sometimes combined with codeine, bromides, etc., if necessary. On account of their depressent action on the heart these drugs are often combined with cardiac tonics such as caffeine and digitalis.

Artificial pyrexia.

Is fever produced by diathermy and physical means the same as fever occurring from infections. The answer is in the negative. From the strictly physiological standpoint, the majority of evidence points towards the two being entirely different, with the exception of temperature or heat, which they both share in common. There are some findings, for example, in the urine and sweat, which are similar, and the clinical interpretation of conditions and results add further to this conception. There is a marked increase in basal metabolism in artificial fever, and possibly much of the benefit comes solely through this source, since its application is nearly always in diseases which at that time are not running temperatures of their own accord. Possibly the increased metabolism and circulation alone, hastening absorption and elimination of their by-products can be just as important a factors. From the physiological point of view, hyperpyrexia produced by physical means is not synonymous with spontaneous fever produced by disease. Hyperpyrexia induced by malaria, chemicals, foreign proteins, etc. is also different from that produced by physical means. The role played by heat retention in combating disease might share a common denominator in all three types of fever. The heart rate is subject to changes in skin temperature more definitely than to changes in body temperature.

5. Effects of Heat (Heat Stroke)

If heat loss is prevented, the body temperature rises, *e.g.*, if a normal man is immersed in a hot bath up to the neck the body temperature rapidly rises from 37°C. to 38°C. (98.4°F. to 100.4°F) or to 40°C (104°F) or even higher, according to the temperature of the bath. This is what sometimes happens in those living in hot climates and heat stroke is the result. The most common form of heat stroke is what is commonly known as 'a touch of the sun.'

The milder cases are termed heat exhaustion. It follows generally after an individual has been exposed to the sun and glare. There are three forms: (1) Mild form. The patient suddenly becomes acutely sensitive to heat and feels weak; there may be nausea, headache, dizziness, staggering pain in the limbs, thirst and sleeplessness. The temperature may or may not rise. He may either collapse in the sun or may drag himself into the shade before collapsing. The pulse is at first rapid and weak, the skin is clammy, the respirations are shallow and hurried, the pupils are dilated and the temperature is normal or subnormal. The mortality in this type is very low, but the patients often recover slowly. *Mild form.*

(2) Severe form. In this form the temperature is considerably or extremely elevated. This is frequently fatal.

(3) Heat cramps.—Here cramps of muscles occur.

(1) Hyperpyrexia

The symptoms start as in the mild form but consciousness is lost early, the skin becomes dry and hot and the temperature rises very rapidly. The pupils are dilated, the pulse becomes rapid and full, and the breathing is deep at first but later shallow and finally of the Cheyne-Stokes type; the pupils become contracted and the conjunctiva injected. The urine and stools may be passed involuntarily, there may be muscular twitchings and rocking of the head. Epileptiform convulsions may occur and death follow. The sudden onset is usually preceeded by a 48 hour period of non-sweating.

The temperature usually is between 99.1° to 104.7°F (37.3° to 40.4°C.). In severe forms the body temperature rises rapidly from 104° to 111.2°F. (40° to 44°C.), rarely to 114.8°F (46°C.). If the temperature does not rise beyond 111.2°F (44°C.) recovery is possible under vigorous hydrotherapeutic treatment. When the temperature exceeds this limit recovery is rare, the patient as a rule becoming comatose, pale, livid and cyanotic. A dry, cool or pale skin indicates that the maximum heat dissipation is not taking place and shows that the normal mechanism of heat regulation has become deranged. Among the most common causes of sunstroke are continued high temperature with high humidity (exercise in warm weather with unsuitable clothing). Heat prostration is not uncommon among soldiers after long marches in full uniform. It also occurs in those individuals whose regulating mechanism against excessive heat is less perfect. Alcohol is an important predisposing factor because it lessens the effectiveness of the regulating mechanism by depressing the heat regulating centres and paralysing the cutaneous vessels. When the patient's temperature is brought down by vigorous hydrotherapeutic measures he may show continuous fever for days or even weeks after the acute hyperpyrexia has subsided. During this time the body temperature is extremely sensitive to external influences, and for years afterwards these patients may show increased sensitiveness to heat. All these factors show that heat-stroke is usually associated with and is followed by a marked disturbance in the heat regulation of the body, the centres which regulate the heat loss being deranged. *Heat prostration.*

Power of acclimatization to heat differs in different individuals. Persons with organic disease of heart and kidneys and such conditions as hyperthyroidism, ichthyosis, etc., stand heat badly and should avoid exposure to it. In people coming from temperate climates physiological adjustment takes place in 2 or 3 weeks. Hours of work should be so adjusted that least physical exertion is done in the hotest part of the day. Sedentary workers should take exercise during cool hours and rest during hot hours of the day. Starches and sugars, which *Prophylaxis.*

are heat producers, should be restricted. Alcoholic beverages should be forbidden till sundown. Water and salts should be freely taken. In labourers who sweat freely, two teaspoonful of salts with plenty of water should be taken and in sedentary workers one teaspoonful daily.

Clothing should be light and loose and of porous material; this promotes absorption of sweat and favours evaporation of sweat. If worn in the sun, it should be white or light in colour. Helmets should be worn to protect the head from the sun rays. Air conditioning prevents acute heat effects and adds to comfort.

Ultra-violet rays of the sun are not so important as the longer heat rays which have great penetrating power.

Treatment.

In mild cases if the patient is put in a cool place and is given drinks of cold water, he often improves. If the pulse remains weak give a diffusible stimulant such as half a drachm each of aromatic spirits of ammonia and spirits of ether, or an injection of camphor in oil. Sometimes in these cases the temperature falls to much below normal and it is necessary to apply heat to the body, and hot drinks have to be given. The patient should be carefully watched as a sudden rise of temperature may occur.

When hyperpyrexia is present such stimulants as aromatic spirit of ammonia caffeine, or camphor in oil should be given at once. Every effort should be made to bring down the temperature as soon as possible. For this purpose the patient is put in a bath of water at 50°F (10°C.) and he is kept there till the rectal temperature falls to 102°F (38.8°C.). When this temperature is reached the body will probably continue to lose heat in favourable cases after removal from the bath, even sometimes to below normal. While the patient is in the bath his body should be vigorously rubbed because unless hyperæmia of the skin is produced by this procedure the overheated blood will be driven to the internal organs by the contraction of the blood vessels. Rubbing the body with ice or placing the patient in a sheet wrung out of iced water are less efficient than the bath. If possible put patient in an air-conditioned room at temperature of 65°F with low humidity. If plethora and high blood pressure, remove 200 to 500 ccm of blood by venesection.

An enema of 2 or 3 pints of ice cold water is often very useful. When ice is not available the heat should be abstracted from the body by evaporation of water. This is done by spraying water from a fine nozzle on to the stripped surface of the body, a current of air being maintained by hand or electric fans. Equally good results are obtained by wrapping the patient in a sheet wrung out in water and putting him under a fan. Tepid water by this method will remove as much heat as ice cold water. This method is very effective and is now extensively used in hospitals. When the rectal temperature (it should be frequently taken) has reached 102°F (38.8°C.) the evaporation is stopped. Cessation of sweating is a sign of impending recurrence. Such patients should at once be covered with a moist sheet and put under a fan. Once the artificial perspiration is established recurrence is often averted. To combat dehydration give fluids freely. Intravenous injection of salt solution with 5 per cent glucose are beneficial. When cramps are present give salt solution freely. Light chloroform anaesthesia may be used to control convulsions. Lumbar puncture may be necessary if cerebro-spinal fluid pressure is increased. If malaria is suspected injections of quinine should be given. Morphine should not be used.

(2) Heat Exhaustion

Symptoms.

Heat exhaustion is also known as heat prostration or heat syncope. In this condition there is little if any, rise of temperature. Predisposing factors are fatigue, alcoholism, debilitating diseases, chronic circulatory disorders, etc. Symptoms develop rapidly or even suddenly. There is nausea, giddiness, weakness, inability to stand or walk; the blood pressure is low, pulse small, weak and rapid, pupils dilated and skin moist.

Mild cases react well to rest alone, but death may occur from failure of circulation, the condition resembling shock. Prognosis is favourable if diseases of heart and kidney are not present. It is frequently the cause of death in the old and debilitated.

Treatment.

The patient should be put in recumbant position in a cool place, with free circulation of air. If temperature is sub-normal wrap in blankets and apply hot water bottles to feet. Cold water containing 1.3 gm (20 grs.) of sodium chloride in a quart of water to be given. Hot tea or coffee or aromatic spirit of ammonia may be given. Strychnine by injection is useless. Digitalin may be given but morphia is contraindicated.

(3) Heat Cramps

Heat cramps, also known as fireman's cramps, stokers cramps and miners cramps.

This is the type of heat exhaustion seen frequently in labourers working in engine rooms or in deep mines where the temperature is high. The patient gets muscular twitchings for a time and is then siezed with violent painful cramps principally of the abdominal muscles. The spasm may involve other muscles and may become so general as to resemble an epileptic attack. Other symptoms are nausea, dizziness and stupor, the patient being usually pale and perspiring. The pulse is rapid but strong and the temperature only slightly above normal. The condition is believed to be due to loss of chlorides to below normal by excessive ingestion and excretion of water.

Prophylaxis.

Good health should be maintained with nutritious diet and adequate rest. Workmen sroould be provided with abundant supply of drinking water and one gram tablets of sodium chloride. Concentrations of 0.1 to 0.15 per cent in drinking water are recommended, as when taken cool such water has no saline taste and quenches thirst. Stronger solutions increase thirst. Stokers in ships often take sea-water and miners in England add salts to their beer for this purpose. New workers should be specially protected in the beginning and physical exertion gradually increased.

Treatment.

This consists in rest and restoration of sodium chloride content of the serum to the normal level. This is best attained by intravenous injection of normal saline to which sodium bicarbonate or glucose may be added. Half to one litres may be given during the first six hours and repeated if necessary. Usually cramps stop after the first injection. Injections are given till the patient can take salt by mouth in form of tablets or normal salt solutions. Dose of salt is one tablet (1.0 gm. each) every hour during day time till 15 tablets are given. Sufficient fluid should be given to compensate for loss by sweat.

In addition to the various causes of hyperpyrexia already mentioned, there may be certain other types of fevers, the etiology of which is imperfectly understood. Low persistent fever or an evening rise of temperature may occur in tropical climates, in persons who are run down or have recently suffered from a prolonged attack of fever. This is probably due to the heat centres not acting effectively and can be controlled by sending the patients to a cool climate such as a hill station.

(4) Prickly Heat

(See under skin diseases). It is a superficial dermatitis in which there is cystic dilatation of sweat gland ducts due to occlusion of their openings. It is associated with high and humid temperature and is accompanied by itching and burning. It is commoner in the obese or stout individuals. It is severe in debilitated and weak individuals. There is acute superficial inflamation of the sweat follicles which may become confluent. Secondary bacterial infection may occur. The condition may last througrout the damp season in the tropics. Keep the skin as dry as possible with dusting powders. Avoid tight underclothing.

CHAPTER VIII

DIET AND DIETETICS IN THE TROPICS

FOOD AND ITS CONSTITUENTS: AMOUNT OF FOOD REQUIRED IN HEALTH, MINERAL CONSTITUENTS, WATER, DIETETIC SOURCES OF THE MINERAL CONSTITUENTS—THE INDIAN DIETARY: MILK PRESERVES—ADAPTATION OF DIET IN TROPICS—DIETETIC ERRORS IN TROPICS: ALCOHOL, DIETETIC ERRORS OF INDIGENOUS RACES—DIET IN THE TROPICS: DIET IN DISEASE, DIET IN PREGNANCY AND LACTATION, DIET IN INFANCY AND CHILDHOOD, INVALID DIET IN TROPICS—DEXTROSE (GLUCOSE): PHYSIOLOGICAL CONSIDERATIONS, MODES OF ADMINISTRATION, GLUCOSE (DEXTROSE) IN THERAPY.

The importance that the Indian patient attaches to his diet is not fully appreciated by Western practitioners. The Hakims and Kavirajes lay great stress on the subject of diet. In Susruta, the well-known book on Hindu medicine, it is said, "The physician that does not know the principles of dietetics cannot cure disease." This idea is ingrained in the minds of Indian patients and unless proper attention is paid to this aspect, patients are not convinced about the utility of a particular line of treatment. It is surprising, however, how few physicians practising Western medicine pay any special attention to this important subject. We have, therefore, thought it necessary to give a chapter on this important subject.

1. Food and its Constituents

Food is essential for the maintenance of the body processes. The human body can be compared to a machine, the fuel for which is supplied by the food that we take every day. Food provides the materials for growth and repair of the fabric of the body. It supplies materials which can be oxidised in the body, with the result that the energy set free by oxidation may be used in performing work and in producing heat. It also supplies the body with substances (catalysts, food hormones, vitamins) which though present in almost inconceivably minute amounts, control body function. Food, therefore, is responsible for the conservation of the material of the body, the maintenance of its output of energy and for the regulation of its functions.

Nutritive constituents.

On chemical analysis, all foods resolve themselves into the following proximate principles usually known as the 'nutritive constituents.'

1. Proteins, *e.g.,* albumin, myosin, gluten, legumin, gelatin.
2. Carbohydrates, *e.g.,* sugar, starch, dextrin, glycogen, cellulose.
3. Fats, *e.g.,* suet, lard, olive oil, butter, fat.
4. Mineral matters, *e.g.,* common salts and compounds of calcium, iron, iodine, potassium, magnesium.
5. Extractives and flavouring materials.
6. Vitamins.
7. Water.

Functions of food.

The various functions of food are fulfilled by the different groups in different measure. The first function, that of building up and repairing the tissues, can be fulfilled by the proteins, the mineral matter and the water. It is true, of course that glucose, one of he carbohydrates, enters into the construction material of all nuclei, of the mucin of mucous cells and of cartilage; while substances such as lecithin with a large portion of their molecules consisting of fatty acid derived from fat, appear in every cell in the body. These substances, however, are almost incidental to the structure of the body which in the main consists of protein, of mineral matter and of water. None of these is sufficient by itself. The second function, that of serving as a source of energy, is mainly fulfilled by the fats and carbohydrates, though we have to recognise that a fair proportion of the energy output of the body may be derived from the oxidation of the proteins. The third function, that of regulating the processes of the body, is fulfilled

by mineral matter and by the vitamins. It is known that muscles (*e.g.*, heart muscle) will not contract in the absence of carefully adjusted amounts of mineral matter. Bone and teeth are irregularly and incompletely formed if in addition to the essential calcium and phosphorus in organic or inorganic combination, there be little or none of vitamin D, in the food. Many other of the processes of the body depend upon the supply in the food, of minute amounts of mineral matter and vitamins.

The relative value of foods.

Physiological opinion has undergone considerable changes during the last century with regard to the relative values of proteins, carbohydrates and fats. Liebig thought that proteins were the chief producers of muscular energy while carbohydrates and fats merely acted as a fuel and maintained the body temperature. This view can no longer be maintained. It is largely a matter of indifference to the cells of the body whether they draw their supplies of energy from protein, fat or carbohydrate although they can get it more readily from protein and carbohydrate than from fat. The maintenance of body temperature is a natural outcome of the metabolic activities of the cell and will be carried on as long as the cells are alive, irrespective of the nature of the food supplied. Tissue repair, however, is a much more important process than heat production and cannot be maintained without the presence in the food of protein, mineral matters and water. The proteins present should be complete proteins, *i.e.*, contain within them all the essential amino-acids; incomplete proteins such as gelatin cannot by themselves take the place of complete proteins in the diet.

The functions and the parts played by the different proximate principles can, therefore, be stated in tabular form as follows—(Hutchison and Mottram) :—

Tissue formers and repairers.	Work and heat producers.	Regulators.
Complete proteins. Incomplete protein in the presence of complete proteins, Mineral matters. Water.	Complete and incomplete proteins. Fats. Carbohydrates.	Mineral matter. Vitamins.

It will be observed that the proteins appear in two categories out of three. They are thus of immense importance in the diet. A diet may be predominantly protein and yet life may be maintained thereon. Without protein, life is impossible as the daily wear and tear of tissue protein must be made good.

From the point of view of physicians, a knowledge of the composition and the nutritive values of foodstuffs in common use is of prime importance. There are methods by which one can judge the relative merits of these food materials.

Chemical analysis.

Ordinary analysis tells how much protein, fat and carbohydrate, are contained in hundred grammes of the food and from this we can form an idea of the value of a particular food as a source of building material or energy.

Calorie value.

The amount of heat which a food yields on complete combustion may be taken as a measure of its value as a source of energy to the body. The unit of heat production is expressed in *calorie* which is the amount of heat required to raise the temperature of 1 gramme of water through 1°C. This is the small calorie. For convenience in measuring the amount of heat of foodstuffs, the large or kilo-calorie is used, *i.e.*, the amount of heat required to raise 1 kilogramme (or 1 litre) of water 1°C (or 1 pound of water 40°F). The determination of the caloric value of a food, therefore, gives an indication as to its value as a source of energy. The heat values of 1.0 gm each of the following are:—

Fat	9.3	Calories. per gm.
Carbohydrate	4.1	Calories. „
Protein	4.1	Calories. „

It will be seen that the caloric value of fat is nearly twice that of protein and carbohydrate. The caloric value of a food, therefore, is greatly affected by its fat content.

The following is the number of calories yielded by the complete combustion of one gramme of the various foods according to Hutchinson:—bacon, 8.86; butter 8.60; fat goose, 4.95; fat pork, 4.12; fat mutton, 4.03; rice 3.51; peas, 3.31; fat beef, 3.27; maize 3.7; coarse white bread, 3.03; wholemeal bread, 2.78; fine white bread, 2.74; cheese, 2.4; eggs, 1.59; lean beef, 0.98; apples 0.59; spinach, 0.34; oranges, 0.33; strawberries, 0.32; lettuce, 0.20.

Burroughs and Wellcome have compiled the following useful table:—

COMPOSITION AND CALORIE VALUE OF PRINCIPAL FOODS.

30 grammes (1 oz.)	Protein Grammes.	Fat Grammes.	Carbohydrate Grammes.	Calories.
Bread —	3	—	16	80
Butter	—	25	—	225
Meat (uncooked, lean)	6	3	—	50
Meat (cooked, lean)	8	5	—	75
Egg (one)	6	6	—	75
Cream, 20 per cent	11	6	1	60
Cheese (Cheddar)	8	10	1	125
Milk	11	1	1.5	20
Potato	1	—	6	30
Peas (dried) Beans (haricot)	7	0.5	17	100
Celery, Cabbage, Cauliflower	0.5	—	0.3	3.2
Lettuce	0.5	—	0.2	2.8
Tomatoes	0.5	—	0.7	4.8
Fish, Codmhaddock (cooked)	6	—	—	25
Bacon	5	15	—	155
Oatmeal, dry-weight	5	2	20	120
Broth	0.7	—	—	3
Small orange or half grape fruit	—	—	10	40

Physiological test.

It is not enough that food should contain a considerable proportion of protein, carbohydrate and fat and should be capable of yielding energy on oxidation. It must be of such a nature that it is easily digested in the alimentary tract and absorbed more or less completely into the blood. There are many substances, *e.g.*, saw-dust, agar, hoof parings which are complete foods so far as their chemical analysis and physical properties of heat production are concerned but which nevertheless are not digestible and cannot serve any useful purpose to the body mechanism. In giving opinion on the qualities of a particular food, therefore, several factors have to be taken into consideration.

Digestibility.

(*i*) *The digestibility of foods.* By digestibility, the biochemist means foods which are rapidly disintegrated in the body under the influence of ferments.

Satiety value.

(*ii*) *The satiety value* or satisfying power of foods. This is a physiologico-psychological quality in which foods differ from one another and is a property which cannot be properly explained. Several factors are at work in giving satiety value to a food and of these the duration of stay of a food in the stomach and the stimulation of the gastric juice are probably important. Fatty foods such as milk, butter, eggs, etc., have a high satiety value while 'lean' fish, bread and green vegetables have a low one.

Absorbability.

(*iii*) Absorbability of the different constituents of various foods is another important factor in dietetics. Of the three chief nutritive constituents, the proteins are the best and most completely absorbed. Animal proteins (milk, eggs, meat) are much better absorbed and the nitrogen loss is much less than in the case of the vegetable proteins (carrots, potatoes, peas, etc.). Compared with the proteins, fats are apparently very incompletely absorbed. This is probably due to the fact that fat which is fluid at the body temperature is more easily absorbed into the blood than one which remains more or less solid. The carbohydrates are more completely absorbed than any other nutritive constituent of the food. Sugar probably never fails to enter the blood to the last grain and even starch only reappears in the fæces when taken in a form specially difficult of absorption, *e.g.*, in green vegetables. Hence foods which consist mainly of carbohydrates, such as rice, leave on the whole, less solid residue in the intestine than any other.

Roughage.

(*iv*) *Roughage.* Though the absorbability is an important guiding principle in the choice of foodstuffs, it is not always an advantage to prescribe a diet which is completely absorbed and does not leave any residue. To ensure the proper action of the bowels, the

intestines apparently seem to require a definite amount of ballast or roughage as we now popularly term it. This is particularly true of herbivorous animals which, if fed upon a diet which leaves little or no residue, suffer from affections of the intestines which may prove fatal, whereas such effects can be avoided by adding to the food any material which leaves behind an unabsorbed residue. That this is universally true of man, whose intestine is shorter in proportion and whose food is not strictly vegetable, has been very largely accepted by modern scientists.

Biological value.

(*v*) *Biological value.* The term biological value is applied to proteins only and its importance in dietetics has only been recently appreciated. By biological value is meant that property of proteins by which they can replace the wear and tear of the body. Thus when it is said that the proteins of milk have a high biological value or those of wheat have a low biological value, it is meant that milk proteins can supply the requisite material for growth at a lower figure for intake than can those of wheat. Proteins of poor biological value have, therefore, to be supplied in larger amounts to counteract the body catabolism than proteins of higher biological value. The biological value of proteins has been expressed in figures. If 100 parts of body protein can be replaced by 100 parts of a food protein then that food is said to have a value of 100. If however 100 parts of it replace only 60 parts of body protein, then it is said to have a value of 60 and so on.

In ordinary dietetics we usually deal with proteins of mixed sources and the quantity consumed is usually sufficient to meet the tissue waste and, therefore, the question of 'biological value' of special proteins does not arise. In the prescription of invalid dietary and under circumstances where life has to be maintained with the minimum quota of protein, the selection of the proper proteins becomes very important. If a protein lacks in the amino acids (lysine, arginine, tryptophane, tyrosine), which are essential to the manufacture of human protein, that protein is absolutely useless to the body economy. For example, gelatin has no tryptophane, no cysteine and very little tyrosine in its composition. Zein, a protein derived from maize, is devoid of lysine and tryptophane. On the whole it has been found out that animal proteins have good supplies of the amino acids in their molecules suitable to the body mechanism while vegetable proteins have considerably less. It, therefore, follows that in nutrition, animal proteins are more valuable than vegetable proteins. Further, it has been noted that proteins of high biological value raise the biological values of other poorer proteins when taken with them. The function of the cheese, eggs, fish, meat and milk proteins which we take in our diet is to render the vegetable proteins of wheat and other cereals and the pulses of greater value in nutrition than they otherwise would be.

(1) Amount of food required in health

Proteins.

A good deal of work has been done by physiologists to determine the optimum quantities of the three proximate principles of food, the proteins, carbohydrates and fats, required by human beings for the maintenance of health and efficiency. No definite agreement applicable to the human race as a whole has yet been reached. The body is remarkable in adapting itself to changes in diet and it would be wrong to apply any hard and fast rules as to what the relations of proteins, fats and carbohydrates should be. As proteins supply the building material to the body and as a healthy man is always doing a moderate amount of muscular work, the protein quota in food should always be considerably above the loss sustained daily, if a proper balance and growth of the body is to be maintained. It has been assumed, as a result of a large number of observations, that the protein in the diet of an average man should not fall below 80 grammes a day and should contain sufficient quantities of good protein (*i.e.,* of animal origin) to supply 5 per cent of the total 'calories.'

Carbohydrates and fats.

The carbohydrates and fats in the diet supply the energy required by the body. As far as the demand of the cells for energy is concerned, it is a matter of indifference how much of the total energy required is obtained in each of these forms. To the digestive organs, however, it is by no means a matter of indifference. If all the energy not provided from protein, were to be obtained from carbohydrate, it would mean that a large bulk of food must be consumed, which would not only overload the stomach and intestines but would also be prone to undergo fermentation. If, on the other hand, fat be adopted as the exclusive source of energy, the limits of fat absorption would be overstepped and nausea and diarrhœa produced. The absorbed fat may cease to be normally metabolised, giving rise to ketosis. Exclusive fat or carbohydrate food, therefore, is not good for the system, though theoretically the required amount of energy can be easily supplied by any one of them individually. The proportion of fat to carbohydrate may, however, vary within a wide range depending upon environment, habit, etc. Thus the Eskimo takes twice as much fat as carbohydrate because his environment does not

produce plant products; the Indian takes much more carbohydrate than fat because cereals and other articles rich in carbohydrate constituents can be grown easily. It has been estimated that in an optimum diet for persons living in temperate climates, 20 to 35 per cent. of the caloric requirement should be derived from the fats and 55 to 60 per cent from the carbohydrates.

Calories necessary.

As a rule, it is appropriate to assume that a man (or woman) who leads a quiet life at home with little exercise requires about 2,500 calories; that if he is engaged in a sedentary occupation, 3,000 calories are required; that if he engages in a moderate amount of exercise or is a labourer doing light work, he can get along on 3,500 calories; and that if he does hard work, 4,000 calories or even more are necessary. Such rough estimates are easy to remember and, while liberal, are sufficiently accurate for most purposes.

According to the British Ministry of Health, the diet should contain 3,400 calories with 50 gm of proteins; the British Medical Association Committee lay down 3,000 calories with 37 gm of protein.

Nutrition committee report.

The first part of the report of the expert Committee on Nutrition states that 2,400 calories net a day are adequate for an adult man or woman living an ordinary everyday life in a temperate climate and not engaged in manual work. This allowance is to be supplemented for various grades of muscular activity. For light work it is estimated that an additional 50 calories per hour of work is needed; for moderate work, 50 to 100 calories; for hard work, 100 to 200 calories; for very hard work, 200 calories or more. For pregnant women 2,400 calories are considered sufficient, and for nursing mothers 3,000. For infants energy requirements are assessed on the basis of 100 calories per kilo of body weight for the first three months of life; 90 calories from three to six months; 80 to 90 calories for six to twelve months. Further, it is suggested that the activities of girls from the age of 7 upwards and of boys from 7 to 11 years should be looked on as equivalent to light work; and those of boys from 11 to 18 years as equivalent to moderate work. As a general recommendation it is suggested that the protein intake of adult should not fall below 1 gm of protein per kilo of body weight, and that a part of this should be from animal sources. Some animal protein is considered essential during growth, pregnancy, and lactation. In the second part of the report, on mineral and vitamin requirements, the committee states that the most important protective foods are milk and milk products, eggs, and glandular tissues; then come green leaf vegetables, fruit, fat fish, and meat. It is also pointed out that "the increasing habit of large sugar consumption tends to lessen the amount of protective foods in the diet and is to be regarded with concern." For nursing voman, the committee stresses the difficulty of arranging such a diet as will provide calcium, phosphorus, iron and vitamins. In this respect milk and eggs are of great service as they contain calcium, phosphorous vitamins and iron. On general lines the committee is of opinion that, (*i*) variety in diet tends to safety, so long as there is a sufficiency of protective, foods; (*ii*) that lightly milled cereals and potatoes should partly replace white flour (excessive consumption of sugar is condemned); (*ii*) that more milk should be taken at all ages, and that the nutritive value of skimmed and separated milk should be more widely appreciated; (*iv*) that fresh fruit and vegetables should always be included in a normal mixed diet; (*v*) that extra vitamin D should be provided, 'wherever sunshine is not abundant' and that the other vitamins are adequately provided in a diet which contains optimum amounts of protective foods.

Vitamins.

(2) Mineral Constituents

The mineral ingredients of the diet are important building material for the body. This will be evidenced from the fact that the human body contains about 7 lb. of mineral matter.

Mineral requirements.

The chief mineral substances required in the food are sodium, potassium, calcium, magnesium, and iron, along with phosphorous, chlorine, sulphur and traces of sillica, fluorine and iodine. Copper must be added to this list, since recent investigations indicate that it is essential for the haemopoeitic function. Some of these elements are distributed very unevenly, for 99 per cent of the calcium and 75 per cent of the phosphorus is present in the bones, three quarters of the iron is in the red blood corpuscles combined as hæmoglobin and nearly the whole of the iodine is stored in the thyroid gland. Most of the potassium is contained in the cells, whilst most of the sodium is contained in the blood and tissue fluids.

The foodstuffs, proteins, fats and carbohydrates in a pure condition contain only five of the fifteen essential elements, and feeding on a diet free from inorganic substances kills animals within a month and more rapidly than does total starvation. This shows very definitely that the mineral constituents are essential for the maintenance of the fabric of the body.

The mineral constituents cannot supply any heat to the body as they enter the body in a highly oxidised form. They are, however, important in regulating the production of energy in the body. Thus calcium is essential in the production of muscle contraction, whether of the skeletal or the cardiac muscle. Iron is essential for the normal processes of all oxidation and the production of energy by tissue cells, iodine in the production of the secretions of the thyroid gland which govern the body metabolism.

No definite estimate of the quantity of mineral matters required for healthy nutrition can be given. Many of the waste mineral matters of the body are excreted by the intestine, and there is no means of telling what proportion of these has merely escaped absorption, and how much has been excreted from the blood after playing a part in metabolism. A rough estimate of the mineral requirements of the diet in grammes per day is given below (Hutchison and Mottram).

Phosphoric acid	3 to 4	Calcium	0.4 to 1
Sulphuric acid	2 to 3.5	Magnesium	0.3 to 0.5
Potassium oxide	2 to 3	Chlorine	6.0 to 8
Sodium oxide	4 to 6	Iron	0.015

In the ordinary mixed diet, the amount of mineral matter present is about 20 grammes and, therefore, is usually sufficient for all the needs of the body. Most of the minerals are in a state of organic combination. Thus both calcium and phosphorus are present in organic combination in milk, iron in meat, sulphur in all protein-containing foods and so on. Minerals seem to be utilised in the body much more easily in organic form than in inorganic form, though there is evidence to show that minerals, like calcium and iron, are quite easily absorbed in a purely inorganic form.

(3) Water

Water is an essential constituent of all protoplasm and a vitally important factor in the body nutrition. The average water content of the body is about 70 per cent and this high figure is absolutely necessary to provide the body with a medium in which chemical actions can occur and osmosis and diffusion can effect the necessary transference of chemical compounds through the tissue membranes. It is thus of much greater importance than the ordinary food stuff and is second only to oxygen regarding the proper working of the complex biological processes inside the animal organism. Since there is a constant loss of water from the body by the kidneys, lungs, sweat glands and bowels, there is a tendency to a greater concentration of chemical compounds in the tissues; but before this can reach a dangerous concentration, the sensation of thirst prompts a replacement of water. This delicate reaction to concentration of the tissue is sufficient to help maintain near its optimum, the balance between water intake and output.

Absorption.

Water may be absorbed into the system through various channels. It is slowly absorbed from the stomach where it also delays the absorption of other substances. It is absorbed readily from the small and large intestines and in these it favours the absorption of substances dissolved in it. In health the absorption mainly takes place from the upper part of the small intestines, the colon only absorbing two-thirds of the water that enters the cæcum. Through the normal intact skin, water is not appreciably absorbed, the outer layer of the mammalian skin may slowly absorb a little quantity, but the passage of significant amounts inwards through the skin does not occur. When ingestion becomes impossible, it is difficult to supply water fast enough through other channels. Rectal administration and parenteral infusion are all inadequate.

The excretion of water occurs mainly by the kidneys, the lungs, the skin and the intestines; and there are many factors which modify the rate of excretion to a considerable extent. Thus if water is taken hot, it favours diaphoresis; if taken in excessive quantity it may lead to diarrhœa and be rapidly eliminated. If it is taken before food, almost an equal amount will be promptly excreted by the kidneys; if it is taken with or soon after food, it may be retained.

Rate of turn over.

The actual rate of turnover of water everyday by the various organs is very interesting. Some of the organs and body fluids such as the salivary glands, stomach, intestinal walls, pancreas, liver and lymph return the water that they drain from the blood. The minimum represented in this way is 3,700 ccm, a more liberal estimate is 9,800 ccm. In the case of the kidneys, the colon, the skin with its insensible loss, the sweat glands and in women the mammary glands, the figures for actual total loss of water from the body range from a minimum of 1,050 ccm to the liberal figure 7,800 ccm. These calculations permit one to estimate the total daily water turnover at something between 4,700 and 17,600 ccm, representing an acaual possibility of more than four gallons.

Water intake.

The body receives water from various sources—ingested fluids, water contained in solid foods and water produced from the complex chemical processes of metabolism. Water taken along with meals has beneficial effects upon all the digestive organs, provided, of course, the food is well masticated. It aids the digestive action of the saliva, and helps the flow of the gastric as well as the pancreatic juice. It secures better absorption of food, and lessens the putrefactive process of the large intestine.

It would be of special interest in this connection to go into the quantity of water taken daily by the people of the tropics. The various climatic influences demand a more liberal intake of water owing to a comparatively high loss of water from the body due to constant and profuse perspiration.

Saderstrom and DuBois give the data regarding the intake and output of water in the daily life of an average normal individual.

Water intake:—

Drinking water	300 gm
Water in coffee, milk, soup, etc.	580 gm
Water in solid food	720 gm
Water from oxidation of 100 gm of protein	41 gm
Water from oxidation of 110 gm of fat	118 gm
Water from oxidation of 244 gm of carbohydrate	135 gm
Total	1,894 gm

Water output:—

Water excretion.

By kidneys	750 gm
By bowels	300 gm
By skin and respiratory passages	700 gm
Total	1,750 gm

Amount retained in the body	144 gm
Gain in the body-weight	100 gm

In the tropical climates the figures of both the intake and output are considerably large. Dehydration may result from low water intake or excessive water output, such as may occur in hot climates. Sensation of thirst normally indicates dehydration first, but under certain pathological conditions it may proceed to a dangerous degree before it can be detected. The superficial signs of lack of water are dryness of skin, shrinkage of subcutaneous tissue and diminution in the quantity of urine. In order to find out whether there is adequate supply of water to the body and proper retention, measurement of the body weight is a reliable guiding factor. Most physiologists will agree, however, that all measurements of the state of body with respect to water must be relatively empirical until the mechanism of regulation is better understood.

(4) Dietetic sources of the mineral constituents

Milk and eggs are the commonest sources of calcium, milk containing 1.5 gm of lime in every litre. Cheese is very rich in calcium but is not largely consumed in India and probably not in other tropical countries. Foods poor in calcium are meat, fish, bread, fruits and potatoes.

Calcium.

An infant requires about 0.3 gm (5 grains) of lime daily. The adult, owing to cessation of growth of the bones, requires relatively less (0.4 to 1 gramme). During pregnancy the calcium need of the mother is high for it has been shown that in the last 3 months of pregnancy the fœtus requires about 0.3 gm of calcium a day. The importance of milk and eggs as foods for growing children and during pregnancy and lactation will be apparent from these facts

Magnesium.

Magnesium is usually present in foods in the same proportion as calcium. Milk also contains magnesium and meat more magnesium than calcium.

Iron.

Iron is usually present in food in an organic form. It is mainly excreted in the fæces, and hence a definite estimate of the daily amount required by the body is not possible. A normal diet contains about 20 mgm or more of iron and this quantity is sufficient to meet all physiological demands.

The amount of iron in different foods varies widely. For example, the following quantities of iron in milligrammes per 10 grammes are contained in certain common foods.

Green vegetables	20 to 40
Peas, dried	5.7
Milk	0.2
Beef and eggs	3.0
Rice, polished	0.9

From the results available, it may be concluded that red meats like mutton and beef, and yolk of eggs are very rich in iron, as also vegetable foods like rice, potatoes, spinach, wheat. As regards an ordinary mixed diet, it may be said that the amount of iron which it contains is roughly proportional to its richness in protein, for these two constituents tend to run parallel to each other.

An abundant supply of this metal is of special importance to growing children and to women during pregnancy. The new born infant starts life with a fairly rich store of iron from the mother, and as long as its sole food is milk, it lives on an iron-poor diet.

Sodium

Sodium is obtainable from the animal group of foods whereas potassium is contained chiefly in the vegetable group. Sodium is absorbed in the form of sodium chloride and, as is well known, this is consumed to a much greater extent than is ever required by the body. The minimum requirement of these salts is from 1—2 gm per diem but an ordinary mixed diet contains 3 or 4 times this amount and hence deficiency in these salts is not likely to occur.

Phosphors enters into the composition of all cell nuclei and it is abundantly present in the bones and in the central nervous system. Wherever growth is going on rapidly a large supply of phosphorus will be required in the food, otherwise development will be seriously impaired. The phosphorus contained in foods is for the most part present in an organic form, part in inorganic form as phosphates of the alkalies or alkaline earths. There is reason to believe that the organic forms are more valuable for contributing to the growth and repair of the tissues; nucleoproteins (liver, kidney, sweetbread), phospho-proteins (milk, cheese, yolk of egg), lecithin (yolk of egg), and other phosphatids and phytin (hexaphosphoric ester of inosite and is found in bran, leguminous seeds and tubers) are all rich sources of organic phosphorus. Fresh vegetables are also good sources of phosphorus.

Sulphur.

Sulphur is present in organic combination chiefly in proteins.

Chlorine is ingested almost entirely in the form of sodium chloride.

Halogens.

Iodine is present in small quantities in fresh water plants, water and land plants. The amount required in the diet daily is estimated to be about 45 microgrammes for an adult and 150 microgrammes for a child.

Fluorine and *silica* are present in the body in small quantities chiefly in the teeth and bones. Vegetable foods and especially the cereals, are the most abundant available sources.

Healthy diets.

A satisfactory diet therefore, must contain suitable quantities of :—

(*a*) Proteins which should be digestible and absorbable and which should have a positive biological value.
(*b*) Carbohydrates.
(*c*) Fats.
(*d*) Mineral constituents including salts, iodine, etc.
(*e*) Vitamins.
(*f*) Water.
(*g*) A sufficient roughage.

The diet must also be free from poisonous substances. Certain individuals are particularly sensitive to certain articles of diet and these should be particularly avoided to avert the unpleasant and sometimes dangerous anaphylactic symptoms.

Taking a broad view, one can safely regard a diet as being suitable when large groups of human beings who have lived on it for generations, are healthy and long-lived. The Sikhs of the Punjab attain to perfect physical development on a diet which is quite different from that which is adopted by Europeans. There is a school of physiologists who think that people who are predominantly vegetarians are likely to be more under-developed and short-lived than those who eat considerable quantities of meat. Practical experience of Asiatic races, who are mainly vegetarians, however, does not bear out the contention of these physiologists, as some of these races are just as healthy and long lived as the

people of the West who take much larger quantities of animal food. It must not be forgotten that the so-called 'vegetarians' are not truly speaking vegetarians as they usually take milk, which is a product of animal origin. The relative merits of vegetable and meat diet have been set forth by different schools of thought and it will not serve any useful purpose to enter into a detailed description of the points which have been raised in this connection. Whatever may prove in the end to be the best for an ordinary adult, who has great powers of adaptability, there can be no doubt that in the case of children. adolescents and pregnant women, it is far safer to adopt a high protein standard than a low one. No risk must be run of providing too little building material for the growing organism.

2. The Indian Dietary

Common Indian dietary.

It will be interesting in this connection to consider the dietary of Indians and to see how far this diet comes up to the satisfactory standard. The importance that an Indian attaches to his diet is hardly realised by Western practitioners. It is not uncommon to find a practitioner writing up prescriptions for medicines without any directions as to the nature of the diet his patient should take. The patients often remain dissatisfied in this respect and may lose faith in the physician.

The Indian diet has largely been founded on economic principles but also partly on the long experience of climate, on social customs, etc., and is now a sound working principle based on a fight against the poor nature of the soil, and the paucity of rains. Two meals are eaten during the day, the morning meal generally consisting of rice and *dal* (pulses) or unleavened bread and curdled milk; the next meal consists of rice with *dal* or vegetables, or unleaven bread with vegetables or meat curry. In villages, the ryot often drinks diluted milk, or curds. In towns during the hot weather some savoury food is taken during the afternoon. Indians are very partial to fruit and in Northern India fruit is eaten before or between these meals. Meat is rarely taken more than twice a week but amongst Brahmins, Buddhists and Jains, meat is not eaten in any form. The nitrogenous portion of the diet is largely made up by milk and *dals* or pulses. We will now discuss these diets in some detail and try to show how far these items of diet supply the necessary proximate principles.

Carbohydrate

The staple food of the people of India is rice. According to the report of the Famine Commission, out of 191 millions people in the country, nearly 68 millions are rice eaters. In the Punjab, U. P., and Oudh, in Behar, the northern parts of the Central Provinces, and in Gujrat (Kathiawar) the poorer classes live on the millets grown in the rains, and on barley and gram; the richer classes eat principally wheat or rice. In Bengal proper, Orissa, and the eastern portion of Central India, rice is the principal food. In the South, in Bombay and the northern parts of Madras, the two varieties of large millets (*jowar* and *bajara*) form the principal food. In Mysore the ordinary food is the small variety *ragi* but rice and wheat are also taken. Baluchisthan, the North-Western Frontier Province and in the higher Himalayas a good deal of maize flour is also eaten along with a certain amount of rice.

The carbohydrate food of the Indians, therefore, can be divided into two types:—

(A) Rice—in the rice-growing areas of Bengal, Madras, Southern India and in sub-montane areas of Nepal, Kashmir, and British India.

(B) Flour—as unleavened cakes (*chapatties*). The bread is made from wheat in the Punjab, millets over Central and Southern India and from maize in the N.W.F. Province, and Bihar.

Sugary foods. Sugar is obtained in India from the sugar-cane and to a small extent from various palms, *e.g.*, the date. Coarse sugar (*jaggery or gur*) is largely used by the poor classes whereas all sorts of refined sugars (*e.g., chini, misri*) are taken by the richer sections of the population. The Indians are particularly fond of sweet things, and if they can afford it, will consume a considerable amount of sugar during the day.

The variety of sweets eaten are enormous and consist of mixtures of sugar with different preparations of milk, curds, rice, *suji,* flour, cocoanut. Milk is often sweetened with sugar before taking.

Proteins.

(*a*) *Milk and Milk derivatives.* Milk is the most popular protein consumed in India. As a matter of fact cow's milk is the staple food of the ryot in most of the rural districts of India. In towns, most of the milk sold is a mixture of cow's and buffalo's milk well diluted with water. Cow's milk is considered to be strength building, fattening and beneficial in gastric conditions. Buffalo's milk, goat's milk, etc., are also used in different parts of India.

Milk Preserves

The shortage of fresh liquid milk in Great Britain made it important to assess the nutritional properties of other forms of milk. The milk proteins and certain of the vitamins are most likely to be affected by processing. In raw milk the protein efficiency in building body protein may be as high as 90 per cent. The vitamin-A content reaches 150-200 I. U. per 100 ccm from May to December and drops to half that quantity for the rest of the year. The vitamin-D content is low, varying with season and depending on action of sunlight on the cow. The riboflavin content varies with the feed, rising from 100 microgrammes per 100 ccm in the stall feeding period to 150-200 microgrammes when the cows are on pasture. Vitamins-B_1 and C contents are independent of feed the vitamin-B_2 content being 10-15 I.U. per 100 ccm, the vitamin-C content 2-2.5 mgm per 100 ccm. The C content however, falls off very rapidly on exposure to light. In the usual commercial preparations of milk, pasteurized, sterilized, spray or roller dried, and condensed sweetened or unsweetened, there is no loss of vitamin A or D, though in skim milk, fresh or dried, there is of course practically no A or D left. The only effect of pasteurization is to cause a 20 per cent loss of vitamin C and a 10 per cent decrease in vitamin-B_1 value. Sterilized milk shows a decrease of 6 per cent loss of C and a 30 per cent loss of B_1. Spray drying causes a loss of only 5 per cent in the biological value of milk proteins, a loss of 20 per cent of vitamin-C content and 10 per cent of the B content. Sweetened condensed milk of good quality shows only a 5 per cent loss of C and a 5-10 per cent loss of vitamin B. In unsweetened evaporated milk there is a slight decrease in protein value, a 60 per cent loss of C and a 30-50 per cent of B. Skim milk except for the loss of A and D is nutritionally valuable because of its high protein, Ca and riboflavin content. All the various preparations of milk possess very valuable nutritional properties and can be used by the adult population as satisfactory substitutes of liquid milk.

Vitamin.

Dahi or curdled milk is one of the most important milk derivatives. It is nothing but ordinary milk which has been allowed to undergo lactic acid fermentation and coagulates into a creamy white gelatinous mass like jnuket and has a pleasant acid taste. Curdled milk is better borne by the stomach as it has been already coagulated and its thick consistency makes it possible to be broken up by mastication. Its acid taste makes it more refreshing than milk and when diluted it makes a delicious cool drink in the hot weather. Metchnikoff's view of introducing into the alimentary canal bacteria that will inhibit the proteolytic action of the organism of putrefaction by virtue of the lactic acid produced by them, seems to have some support by this universal custom of taking curdled milk. Potcheese (*chhana*) is made by coagulating milk by citric acid, *i.e.,* the juice of the lemons and removing the whey. It must be used fresh as it deteriorates very quickly. Whey (*ghol*) is a greenish yellow coloured liquid with a sweet acid taste. Whey contains lactose and lactalbumin and is devoid of albuminoids so that it is easily assimilated. Buttermilk is the liquid that is obtained after the milk has fermented and the fat has been churned off as butter. It is called *chachh* (U.P.) or *lassi* (Punjab) and is largely drunk in Northern India. Being fat free, it should be useful in hepatitis, jaundice and sprue.

(*b*) *Meat.* Meat is undoubtedly the most assimilable form of protein in the food. The religious practices and social customs existing in India are sometimes great obstacles to meat eating. Thus the Brahmins, Jains and Buddhists are prevented from eating meat by their religion. Generally speaking, meat is used sparingly in India. Beef is used only by the Mohammedans and foreigners in India; mutton is more commonly used by the Hindus. The meats of the domestic fowls and wild game birds are also sparingly used. The Hindus in some parts abstain from taking the meat of domiestic fowls, but are allowed to eat game.

(*c*) *Fishes.* There are namerous species found in the Indian rivers; these vary in quality; some are of fine flavour (*Hilsa, Mrigal, Katla* and other fat fish) while others are poorer in quality (*Koi, Singhee,* and other lean fish). Fish is an important article of food in Bengal where very little meat is taken. Sea fish is taken by certain sections of the population living along the coasts.

(*d*) *The 'pulses' or 'dals'.* The pulses largely supply the nitrogenous constituent of the diet of Indians. Various kinds of pulses are grown all over India and are available at a cheap price. The dals may be taken in various ways, many of them are cooked in the green unripe state as a vegetable. When dried and ripe, the pulses may be cooked as a *puree* with the addition of some ghee. The pulses contain larger quantities of proteins and in many cases make up for the deficiency in foods such as rice.

Ghee. Clarified butter or ghee is the commonest fatty substance taken in the country. Butter is made from milk-fat obtained by churning which mechanically ruptures the albuminoid envelops surrounding the fat droplets and allows them to come to the surface. This butter is clarified by heat and is then known as ghee. Ghee is used by all classes of people who can afford it, in the preparation of vegetables, curries, etc., and enters into the composition of many of their sweets and pastries. *Fats.*

Sesame oil. The oil from the seeds of *Sesamum indicum* (til) is used in some parts. This oil, however, is expensive and is not much used in cookery.

Cocoanut oil is a fixed fatty oil with a specific gravity of 0.892. The bulk of oil is obtained by cold expression from copra. In Southern India especially in Cochin and Travancore, it is used extensively in cooking.

Mustard oil is obtained by pressing the seeds of the mustard (*Sinapis nigra*). It is largely used in Bengal and in other parts for cooking purposes. The oil must be heated at first to destroy the action of the ferment myrosin and to remove the volatile pungent essential oil. *Walnut oil* is largely used in Kashmir for cooking purposes.

Rancid fats have long been recognised as nutritionally deleterious. Mixed with other foods the rancid fats cause destruction of such essential food factors as Vitamins A, D and E, carotene, biotin, pyridoxine, ascorbic acid, pantothenic acid and linoleic acid. Feeding experiments on albino rats have thrown much light on the problem of rancid fats. *Deleterious effects of Rancid fats.*

Two batches of albino rats were fed separately diet containing rancid fat and corn oil. The diets were otherwise perfect and similar. After lapse of a year the rats on food-mixture containing rancid lard were emaciated and later a progressive paralysis starting from the hind limbs developed which became extreme before death. They found that the development of rancidity is more rapid in food mixtures than in unmixed lard and that little or no rancidity develops at 20°F."

3. Adaptation of Diet in the Tropics

Among all the demands which the body makes on its environment that for food is of outstanding importance; correct nutrition may profoundly affect the well-being and the social value of the individual. Considerable attention has therefore been directed to the adjustment of diet for different deficiency diseases and on the question as to how a wellbalanced diet, containing all the proximate principles and other important constituents could be made available to the masses. Caloric value of foodstuffs, which was at one time the basis of dietetic studies, is relegated to the background, and comparatively less attention is being paid to their energy and heat-producing properties. *Protein and fat requirements.*

The adaptation which man is called upon to make in the tropics is primarily one of heat regulation. The problem of diet is intimately connected with the problems of heat regulation, body temperature and metabolic rate. The question of the suitability or otherwise to the tropical climate of the common foodstuffs available in the tropics is not satisfactorily answered. Nature offers fat and protein in the arctic regions and carbohydrates at the equator, and man in these areas has always accepted them as his staple foods. It is not clear as to what is the optimum or ideal to be aimed at with a view to effecting a suitable adjustment to the climate.

The proportion of protein, fat and carbohydrate varies considerably in the diets of different races, depending largely on the type of food available and also upon the customs existing in particular localities. The prevailing diet of the masses in the tropics is derived largely from vegetable sources and carbohydrates generally predominate in it. Nearly a century ago, Chevers thought that the Hindu dietary with a very moderate quantity of animal food was the one most suited for a tropical climate.

The protein requirement of the tropical races has been the subject of intensive investigation and research by a large number of works in India and other tropical countries but no definite conclusions have been drawn. It has been pointed out that in many parts of the world those who consume a diet with high protein content have a better physique and are more virile than others of the same race who, for one reason or another, consume *Biological value of Proteins.*

less protein. In the north of Italy, for instance, where the protein consumption is higher, the physique is better than in the south. The same statement has been made about India although here racial differences may have something to do with the differences. According to McCay 'As we pass from the north-west region of the Punjab down the Gangetic plain to the coast of Bengal, there is gradual fall in the stature, body weight, stamina and the efficiency of the people. In accordance with this decline in many characteristics, there is an accompanying gradual fall in the nutritive value of the dietaries'. McCarrison working in South India confirmed the findings of McCay and concluded that there is a chronic protein starvation in the dietary of the Indians. Recent researches by Aykroyd and Wilson in this country have shown that the main deficiency in the diet is the lack of proteins of high biological value and certain salts, and this defect in the dietary, which can be easily corrected with small quantities of milk, is responsible for the malnutrition and consequently the poor physique of the children. On the other hand, the fact connot be overlooked that the meatless diet of some of the finest soldiers of the Indian army has a low protein content. There is already a considerable volume of opinion that the protein intake in the tropics should be less than in temperate climates. It is well known that protein food, by virtue of its specific dynamic action generates a good deal of heat. The Eskimos, who live in the arctic zone, consume large quantities of meat, sufficient to raise their metabolism over 50 per cent which is an obvious practical advantage. Such a diet in the tropics would be considered unsatisfactory as it would throw an additional burden on the heat-regulating mechanism which is already over-taxed. From this point of view a low protein vegetarian diet seems logical and advisable in the tropics. Recent researches tend to show that there are a number of important factors which deserve special attention in determining the minimum quantity of protein necessary to preserve nitrogenous equilibrium, *e.g.*, the particular protein the subpect is accustomed to take and the ratio of the inorganic bases to inorganic acids available to or formed in the body of the subject. The coefficient of absorption also varies enormously in proteins of animal and vegetable origin. Inhabitants in the tropics usually obtain a large percentage of their protein requrement (70 to 80 per cent) from vegetable sources while Europeans do not draw more than 25 per cent from this source. Vegetable protein is said to have less 'biological' value than animal protein. It is futile to work out an allowance of say 80 gm of protein per day, if a good protion of it is derived from vegetable sources with poor 'biological' value and it may not be assimilated. Then again, a food may show variation in the absorption of its protein content merely because of various methods of cooking. For instance, *dals* (lentils, commonly used in Indian dietary) if not properly cooked lose a lot of protein value. If they are simply boiled until soft, as is usually done and consumed, about 40 per cent of the protein content is lost from non-absorption. If, on the other hand, they are mashed and cooked with special attention to the temperature and water content, the loss is not so great. This also applies to rice which is the staple article of diet in a large part of India. A diet, theoretically perfect, may still be quite unable to supply the physiological needs of the race for whom it is intended. It is therefore of utmost importance that, in assessing the amount of protein in a diet, its source should be known and the computation for protein requirements made, with special reference to the manner in which it is going to be available for final consumption.

Fat-free dietary.

It is a common experience in the tropics to find that the poorest classes live on an almost fat-free diet. The very small amount of fat that they do eat is also often adulterated. Thus, mustard oil which is consumed by quite a large section of the population in Bengal is often impure and contains adulterants; the palm oil of other tropical dietaries is very variable both in fat and vitamin content. That fat is an important constituent of diet has been long known but that it is also an essential constituent which cannot be replaced entirely for instance, by carbohydrates was not realized before. Recent researches have forcibly stressed the fact that fats, if completely excluded from the diet, might lead not only to stunted growth but also to the development of various lesions indicative of malnutrition in the body. The fatty acids in fat are probably quite as important as the fat-soluble vitamins. From these considerations, it is logical to conclude that the almost fat-free diet of the Indian races is an important cause of the general and wide-spread malnutrition. From the point of view of adaptation to a tropical environment a diet comparatively poor in fat has obvious advantages. In the colder parts of the world, where harder physical work can be, and often is, performed, a fairly high caloric intake is necessary and this cannot be supplied without including a generous proportion of fat. A caloric intake of a Canadian lumberman of 9,000 calories, for example, could not be consumed as starch without putting too much strain on the demands of the alimentary canal. Fat is much less bulky than cereal foods and, bulk for bulk, is a much richer source of energy and heat. Besides in the tropics an unduly large layer of body fat—external or internal—is not so necessary as in the cooler regions, which naturally leads to a diminished demand for it in the dietary.

Carbohydrate in the dietary.

Carbohydrates have a definite and important effect on the physiological mechanism of the body. Numerous workers have noted that a change from a fat diet to a starch one involves considerable retention of water and *vice versa*. Some of it is taken up by the stored glycogen and some as extra fluid in the extracellular and probably intracellular fluids. In other words, on a carbohydrate diet, an increased amount of water is available for evaporation and urine secretion in case of necessity. Moreover, carbohydrates are always the first to be metabolized and hence *pari passu* more motile fluid is available while they are being consumed. Experiments on rats on a fat-free diet have shown that these animals consume more water and excrete actually less urine than others with fat in their diet. More fluid appears to be eliminated by the lungs, under such conditions. Further, pulmonary ventilation is greater when carbohydrates are being burnt, owing to a larger output of carbon dioxide per caloric of heat than on a fat diet. This would increase the heat loss by evaporation of water from the lungs. These experiments are suggestive in view of Eijkman's (1924) observations in Java. He found that the Malayan is more economical (physiologically) in his water elimination than the European. Under identical conditions, a European loose 143 gm of water and a Malayan only 105 gm of water by insensible perspiration in a given period. Contrary to expectation, the Malayan had also the smaller volume of urine ,even though he had received extra water, while the European had been deprived of it during the observation. The European appears to waste water as liquid perspiration which does not therefore perform its physiological function, namely, evaporation and heat loss. The problem therefore is how much of this adaptation is racial and how much of it is due to carbohydrate diet conserving the body fluids. The increased urine elimination of the European may have been due to his greater excretion of nitrogenous products which would be much less in the Malayan with his low protein diet. The Malayan, on the other hand, may consume more salt, which under certain circumstances promotes retention of water.

The considerations set forth above are particularly important if we are to construct a dietary suited to the climatic conditions of the tropics. Some medical men, obsessed by the idea that a good dietary must be rich in protein and fat, have advocated the wholesomeness of the protein-rich European dieatry for the tropics, overlooking the fact that whole nations and working classes can develop satisfactorily and lead healthy and mentally productive lives on a diet which is almost equivalent, as far as caloric value is concerned, to the European diet but which is very poor in animal protein. It seems that dietary types like racial patterns cannot be laid down in rigid terms. The dental structure and alimentary canal of man are pre-eminently suited for a mixed diet and he is therefore probably capable of adapting himself to diets varying widely in their compositions. The determination of the minimal animal protein requirements is an interesting subject for further research and it is possible that these depend considerably on climatic considerations.

4. Dietetic Errors in the Tropics

Over-feeding.

It is perhaps no exaggeration to say that the tendency of civilised people, and especially of the upper classes in civilised society, is to eat more than the necessary minimum. A moderate excess of food is probably harmless, if not actually beneficial. For it is well to have some reserve in the body which can be called upon in cases of emergency. This limit, however, is frequently overstepped by the well-to-do people in the tropics. The nature of the diet in the tropics is predominantly of vegetable origin and contains plenty of carbohydrates. The general results of habitual or chronic overfeeding with such bulky carbohydrate food are pendulous abdomen and obesity. The sedentary habit of the majority of people further helps in diminishing tissue catabolism and consequent deposition of fat. Obesity is one of the commonest end-results of overfeeding.

Protein shortage and diet.

In general, bodily activity is reduced in the tropics and the digestive powers are undoubtedly not so vigorous as in cold climates. The European coming out to Eastern countries cannot easily give up the dietary habits and tendencies of a temperate climate. A high protein diet which is wholesome and perhaps a necessity in cold countries may actually prove injurious in the tropics. Proteins have a high specific dynamic action and a diet which includes proteins in excess is naturally a 'heating diet.' In hot weather, the production of excessive heat inside the body may be not only uncomfortable but may be actually injurious.

Further a predominantly protein diet is likely to leave a very imperfect residue as such diet is always better absorbed. In the tropics the general musculature of he body as well as the intestinal muscles are not kept in that tone which is common in the residents of the cold countries and, hence, constipation results. Habitual constipation leads to intestinal stasis and the absorption of toxic end-products of protein cleavage. Gradually the constitution gets undermined, bowel troubles supervene and mental disturbances in the from of neurasthenia, failure of memory, irritability, etc., result. It is also probable that sprue and appendicitis, so commonly seen amongst the Europeans and so makedly absent in the indigenous races of the tropics, been some relation to auto-intoxication from the bowels.

(1) Alcohol

Indulgence in alcohol is another factor which may advantageously be considered under this section. Though alcohol is not an article of food, it is commonly taken as a drink along with food and this is perhaps one of the most important dietetic errors in the tropics. Europeans suffer most from the alcohol habit though in recent times, a good section of the Indian community has also contracted the habit. The plea usually put forward is that alcohol is necessary for the maintenance of health and that without its stimulating influence, no strenuous work can be carried on satisfactorily. Others consider it a stimulant which is necessary in the tropics after a day's hard work. It is also believed to be a protective from malaria, and bowel and other diseases. These ideas have all been shown to be erroneous. Though one cannot deny to alcohol the right to be regarded as a 'food' in the scientific sense of the term, it cannot be regarded as a food of great importance, for although it is able to replace fat and carbohydrate it cannot be converted into these, while its secondary effects on the nervous and vascular systems counteract, to a large extent, the benefits derived from the production of heat and energy by its oxidation. Many writers have produced evidence to show that it is not favourable to the production of healthy mental labour. Alcohol has no influence on physical labour and sustained muscular effort, as is generally supposed. It may even do harm by paralysing the sense of fatigue which is the natural check on excessive exertion.

On the other hand, whenever the proportion of alcohol circulating in the blood becomes greater than the cells can rapidly deal with, it acts as a protoplasmic poison. The brain cells seem to be peculiarly sensitive to the paralysing action of alcohol, so that the brain is the first to show the effects of an overdose and intoxication results. If this overdosage is kept up for a very long time, the highly organised and delicate brain cells would naturally become the seat of various degenerative changes. A certain degree of dulling or even paralysis of the moral perceptions has been noticed in chronic alcoholics by many observers. Apart from its bad effects on the brain centres, the presence of undecomposed alcohol leads to a diminution of the chemical energy of the cells which interferes with the ordinary course of metabolism and may result in chronic disease. The metabolism of fat is most likely to be interrupted and hence alcoholism is a common cause of fatty degeneration.

The excretion of undecomposed alcohol through the kidneys may also act as an irritant and bring about changes in structure and ultimately may produce chronic nephritis. It has injurious effects on the gastro-intestinal tract and particularly on the liver. Alcohol also paralyses the blood vessels and thus interferes with dissipation of heat from the body. It renders people liable to heat stroke and alcoholism is one of the prolific causes of this condition in the tropics.

Relation of alcohol to heat stroke, liver abscess and tropical neurasthenia.

It will thus be seen that alcohol is an unnecessary article of diet in normal health and is not truly beneficial to the system but is actually injurious from the physiological point of view. Alcohol, there is little doubt, is a potent factor in shortening the life and undermining the health of the majority of Europeans in the tropics. Alcohol strongly predisposes to liver abscess, heat stroke and tropical neurasthenia. It makes the habitue a bad subject for surgical operations and lowers the resistance of the tissues to intercurrent infections. Obesity, diabetes and other disorders of metabolism like gout, etc., have all been ascribed to the excessive ingestion of alcohol.

Over-indulgence.

The injurious effects of over-indulgence in alcohol in hot countries cannot be over-estimated. It has been approximately ascertained that from 1 to 1½ ounces of absolute alcohol is about the amount that can be utilized as a food, or completely oxidized in the body in twenty-four hours, though some put the amount at 3 ounces, and consider that in that quantity it is a food.

If we accept the first, that amount of alcohol would be represented by the following quantities of the more frequently used alcoholic drinks. Brandy or whisky (40 to 47 per cent alcohol) 3 fluid ounces; port, sherry, or other strong wines (about 20 per cent

lcohol), 7 ounces claret, hock, champagne, and other wines (5 to 10 per cent alcohol) 5 ounces or a tumblerful and a-half; strong ale (bottled) (10 per cent alcohol), 20 unces, or one imperial pint.

Alcohol is a chemical irritant and the amount of irritation on the nerve endings, nucous membrane and other organs is entirely proportionate to the degree of dilution nd the quantity taken above the amount specified. Alcohol is rapidly oxidized in the ody as result of physical exertion, and under such conditions more can be onsumed without injurious effects than in case of an individual with sedentary habit. While total abstinence may not be essential in warm climates alcohol in moderation may e taken stimulating and restorative effects. It should not be taken during the hotest art of the day or when it is intended to be out in the heat of the sun. *Alcohol an irritant.*

(2) Dietetic Errors of Indigenous Races

The chief defects consist in the excessive bulkiness and low nutritive value of the liets. People who live chiefly on rice often take 30 to 40 oz daily; this, when cooked, orms a very bulky diet which causes distention of the stomach and is incompletely ligested. McCay, who carried out important investigations into Indian diets, made the ollowing observations:—(1) The absorption of proteins from cooked rice is actually liminished when excessive quantities are eaten, for example, 8½ gm were absorbed when 9 oz of rice were eaten daily, but only 6½ gm when 30 oz were consumed. The larger ration does not make up for the poorness of proteins of the diet but rather the reverse, while the excess of carbohydrates upsets the balance of the diet. (2) Certain vegetable sources of protein are unsatisfactory; from a diet of millets, dal and vegetables containing 16 gm of protein in all, only 9½ gm were absorbed, whereas from a diet of wheat containing 16 gm of protein as much as 13 gm were absorbed. (3) Unsuitable methods of cooking and disorders of the alimentary system caused a reduction in the absorptions of proteins. (4) Great variations in physique and fitness were found among the races using different diets. Europeans and Sikhs who absorbed over 0.25 gm of protein per kg of body-weight were far more robust than Bengalis who absorbed only 0.11 gm per kg. The average Sikh diet approaches closely to good European standards: it consists of milk 3/4 pint, wheat 24 oz butter or ghee 2 oz, dal 3 oz, vegetables 6 oz, and meat 4 oz. An unexpected finding was that the low protein absorption of the Bengalis was associated with increased prevalence of degenerative diseases of the kidneys. The reason for this is probably malnutrition of the kidney cells.

The nutritional defects of indigenous populations are easy to discover but difficult to rectify; they are closely associated with poor economic sandards of life, which in turn are connected with social customs, especially early marriage and unrestricted procreation of children. So long as immature boys and girls marry and proceed to have large families before they are able to maintain them, there is no prospect of economic improvement; any increase in the production of food will quickly be neutralised by a corresponding increase in the population. The solution of the problem lies in a rational education directed towards showing the rising generation how to adapt themselves to their environment by later and more provident marriages or by limiting the number of children in some other way. The chief dietetic error which prevails among the richer members of the tropical communities consists in excessive consumption of carbohydrates, especially in the form of easily absorbable sugars. This fault, when combined with lack of exercise, leads to obesity and finally to diabetes.

5. Diet in the Tropics

(1) Diet in Disease

Diet plays a very important part in the treatment of disease in the tropics. Diet may be considered in the light of a therapeutic agent and, like all other remedial agents, has a limited place in therapeutics and should not be considered as a panacea for all ailments. Dietetic treatment is specially important in a particular group of disorders and we will confine our attention to these disorders primarily. The principles of feeding will only be outlined, the detailed regime will naturally have to be laid down by the physicians with special reference to the particular type of requirement.

The prejudices of patients and the superstitious beliefs existing in India sometime make it very difficult for the physicians to prescribe a regular routine

of diet according to strict scientific principles. Thus milk is regarded in some parts of India as injurious to those who have got a cough as it is supposed to increase the phlegm. In some places, it is forbidden in cases of diarrhœa. In prescribing diet these prejudices and beliefs have to be attended to. Individual susceptibility to various foods should also be taken into account.

Diet in fever.

The pathological conception regarding the production of fever has undergone a radical change within recent times. In the time of Hippocrates and long afterwards, fever was supposed to originate from bad humour and intestinal irritation and hence the principle of diet was either to starve the patient or to allow only such bland non-irritating and harmless articles, e.g., thin barley gruel and old wine which will allay intestinal irritation and thereby diminish the range of the temperature. A revolt against this starvation diet was not, however, late in making itself manifest and a second school of thought promulgated the idea that fevers were asthenic diseases and should be treated with liberal feeding rather than starvation. Though this doctrine has never been adopted *in toto* in practice, it must be admitted, in the light of our present knowledge of the metabolic changes in fever, that the plan of 'feeding fevers' is a much more rational and sound plan than starvation diet. Thus it has been observed on many occasions that the free administration of food does not, as was formerly supposed, tend to raise the temperature of feverish patients. Von Hoesslin found that in cases of typhoid the temperature during the days when food was administered was only from 0.11°C to 0.3°C higher than on starvation days. Moreover, the absorption of light articles of diet may go on as perfectly in the febrile as in the non-febrile state. There is, therefore, no reason why a fever patient should not be allowed to have sufficient food up to the limit of his digestive capacity which is greatly interfered with during febrile conditions.

From a study of the metabolic changes, the chief dietetic indication in general fevers, therefore, is to supply a large proportion of protein in the diet to counteract the active protein loss. Though this appears quite a simple matter theoretically, it has been observed that it is impossible to bring about a condition of nitrogenous equilibrium in acute fevers. The noxious action of the toxins on the tissue cells renders them incapable of assimilating proteins, so that the admnistration of protein diet during febrile stages instead of repairing the breakdown, actually does harm by flooding the circulation with protein cleavage products which are toxic to the liver and the kidneys. It is not logical, therefore, to give too much protein ration to fever patients. Carbohydrates and fats are known to be 'fever protein-sparers' under certain circumstances and the tissue breakdown in fever can only be stopped by supplying these in abundance. Then again, fats are not liked by fever patients probably because they are not readily digested. The carbohydrates, therefore, are our mainstay in dieting fever patients.

General scheme of diet.

For purposes of dieting it is necessary to secure such a state of affairs as will coax the appetite, create relish for such insipid food as may be allowed and a clear state of the gastro-intestinal tract. A fluid diet is considered the best by common consent. This evades the necessity for chewing which is rendered difficult by the diminution of the salivary secretion and also relieves the thirst and supplies plenty of fluid to the parched tissues. Milk is the simplest, most accessible and most nutritious of fluid foods and should always form the basis of the dietary. Plenty of fluid drinks may also be allowed. Some workers claim that saline-containing beverages are better than ordinary drinks as these may replace the salts which are rapidly excreted from the kidneys during fever. Such a claim, however, has no real value. The kidneys

have the power of re-absorbing through the tubules the salts necessary for tissue metabolism and if we assume that the kidneys are in a healthy condition, there is no reason to think that the mineral constituents of the cells have drained away through them and that replenishing is necessary through the mouth.

A common mistake in tropical countries, particularly in the summer season, is to allow iced drinks. Though there is no evidence to show that these are injurious in any way, they have been frequently observed to give rise to a congestion of the throat and a distension of the stomach. The excessive consumption of ærated beverages is to be avoided, as tending to overdistend the stomach with gas. The water of unripe cocoanut (*dab*) is a very pleasant drink available in the tropics and may be allowed if the patient cares to have it.

The system requires much less energy to maintain it at a normal level while in rest than when it is carrying on some muscular work. As all the fever patients usually remain in bed, the caloric requirement is also small. It has been estimated that for an adult human being of average weight, nearly 1700 calories are required in the resting condition to keep it in balance. If four pints of milk are consumed in a day, the necessary caloric value is obtained and there is no necessity to worry about the tissue break-down. If, however, this is not taken for some reason or other, the milk should be fortified. The amount of protein in it may be increased by the addition of such preparations as Plasmon or the proportion of fat may be raised by the addition of cream. It is better, for reasons already adduced, to enrich the milk with carbohydrates such as glucose, milk-sugar, cereal preparations like arrowroot, oat flour, corn-flour, barley, sago, sati, rice-gruel, etc. Fruit juices such as those of orange, pomegranate, grapes, pine-apple, pears, apples, jamrook, etc., may also be given in moderate quantities. Sugarcane is a very good thing in India if the patient is not too weak to chew it. It cleanses the mouth and the juice is very refreshing. In addition to these, soups, beef tea, thin pea or *dal* soup or broths may be allowed in the proportion of about one pint a day but these are better avoided particularly when diarrhœa is present.

Use of alcohol in fevers.

Alcohol is undoubtedly of value in febrile states if it is used in moderation and according to the direction of the physician. Its properties as an indirect stimulant to the heart is too well known to need any special mention and in fevers which are asthenic conditions, a stimulant in any form should be helpful. Alcohol reduces body temperature by dilating the blood vessels of the skin and diminishes tissue waste specially the waste of fat and these properties should mark it out as an agent of great value in fever. It is absorbed immediately and the tissues can use it as a ready source of energy.

Alcohol has been largely used in conditions of failing circulation in fever which are characterised by a quick, weak and irregular pulse. In nervous prostration and sleeplessness also it has been highly spoken of when the digestive powers are very weak, the property of alcohol to spare the tissues can also be utilised. In cases of hyperpyrexia, its use has often been attended with very gratifying results.

(2) Diet in Pregnency and Lactation

Diet in pregnancy.

Pregnancy is a natural physiological process, and minor ailments associated with it, such as morning sickness, etc., should not be regarded as pathological. The general health of the average pregnant woman, however, in these days, is poor and she is often considered to be an invalid at such times. A large amount of sickness and disability associated with child bearing is due to our lack of knowledge of the relationship of the nutrition of the mother to the normal functioning of her organs. The serious consequences of a

deficiency in many essential food substances and with a pregnancy superadded may be greatly reduced by judicious dieting. This problem does not affect the mother alone, the growing fœtus also suffers serious results.

The early fœtal growth takes place at the expense of the maternal tissue and there is always an extravagant breaking down of it to enable the fœtus to get its requirements. At a later stage the fœtus derives its nutrition from maternal food. Over and above this, many chemical substances which are essential for its growth are present only in small quantities in maternal organs and they cannot be synthesised by either the mother or the fœtus. A liberal supply of these substances (or their effective precursors) must be from the food the mother takes. Ordinary diets vary widely so far as the energy bearing factors, the proteins, the fats and the carbohydrates are concerned, and yet they may remain compatible with good maternal and fœtal health. The trouble arises, when there is unbalanced dietary and a deficiency of factors essential to proper structure, function and even to life of the mother and of the offspring. Among these are calcium, phosphorus, iodine, iron and vitamins A and D. A pregnant and lactating woman has to supply calcium to the fœtus from her own store. Calcium and phosphorus of different foods are retained by the body to different extent and the retention is greatly influenced by the presence of vitamin D. Cereals such as oatmeals and cereal embryo which are rich in calcium and phosphorus are anticalcifying and along with them a well-balanced amount of calcium-retaining factor in form of milk and cod-liver oil (which ensures a good supply of vitamin D) should be ingested. Cod-liver oil also supplies vitamin A which increases the resistance to infection.

The significance of supplying iron to the pregnant mother is that the fœtus takes away iron from the mother in order to enable it to store iron in the liver to tide over the milk diet period, when it gets a deficient amount of iron. Consequently a failure to supply mother at such a time with sufficient iron will tell upon herself and the child as well. The daily diet should consist of food rich in iron, such as meat, egg-yolk, calf's liver, green vegetables, etc.

Iodine is of importance both to the mother and the offspring. A deficiency of iodine in pregnancy results in cretinism in the offspring and simple goitre or myxœdema may follow numerous pregnancies in a mother. Sea fish or any kind of living matter from the sea and cod-liver oil should be included in the diet.

Suggested diets.

According to Mellanby "the diet of pregnancy and lactation ought to include the following:

2 pints of milk daily.

1 or 2 substantial servings of green vegetables—cabbage, spinach or lettuce daily.

1 or 2 eggs or egg-yolks daily.

An apple or orange or some fresh fruit daily.

Sea fish twice or more a week.

Cod-liver oil may be taken 2 teaspoonfuls daily.

The rest of the diet can be made up as the expectant mother desires."

Green-Armytage recommends for the Indians, the following diet with the addition of radiostoleum and Parrish's food.

Cereals	.....	*Dhenki* (home-pounded) rice; *atta* (whole meal flour) and *suji.*
Vegetables	.....	*Sak* (green leafy vegetables) in all forms except fried *Sabji* (green vegetables, etc.), bean, pumkins, cucumber, brinjal, green peas, squash, cabbage, cauliflower, tomato, etc.
Fruits	.....	All, fresh or stewed.
Eggs	.....	Ducks or hens in any form except fried.
Fish	.....	Mud fish like—*kai, magur, singee.*
Meat	.....	Mutton, chicken, liver, kidney, etc.
Fats	.....	Oils, butter, ghee—sparingly.
Milk	.....	Fresh goat's or cow's in any form—plain, *dahi* or *ghol.* Avoid buffalo's milk.
Sweets	.....	Honey, molasses, jam, jelly, marmalade. No pastry, no cakes.
Fluids	.....	Water ad lib, cocoanut water, weak tea, coffee, aerated water, home-made lemonade or lime juice.

It is important to avoid any excess in the individual articles of diet as well as in the total intake of food. The idea that a pregnant woman needs large quantities of food is erroneous. Excess of fat in the diet should be avoided not only during the early months when vomiting is a troublesome symptom, but also during the subsequent months of pregnancy. Vomiting is due at least in part, to excessive fat intake which must be limited.

Diet and toxaemias of pregnancy.

The position regarding the relationship between diet and toxæmias of pregnancy has been summarised by Mellanby. "It is unfortunate that there is no established clinical or experimental evidence that malnutrition is responsible for those toxæmias of pregnancy which include albuminuria, pre-eclamptic toxæmia, and eclampsia itself. In spite of the absence of such evidence I wish to plead that future investigators of these problems will give some attention to this aspect of the case. The hypothesis that such toxæmias are due to the production of toxic substances by the fœtus and placenta has not been fruitful. On the other hand, there is very indication that they are due to abnormal metabolism and, since so many defects in metabolism are now known to be nutritional in origin, the chances that such a factor is the basis of eclamptic conditions are large."

"The final teaching based on the foregoing facts is that proper nutrition is essential to healthy childbearing and that the diet should include throughout pregnancy and lactation a sufficiency of what are known as protective foodstuffs. When plenty of these are taken, there is every reason to believe that the rest of the diet will take care of itself."

In abnormal cases, the diet can be dealt with under the following headings:—

1. Constipation—It is often benefited by increasing fluid intake and the amount of fruit and vegetables in the diet. It is better to avoid taking purgatives of drastic griping type. Saline aperients or lubricants might be used with good results, if at all needed.

2. Albuminuria—In pre-eclamptic cases the dieting should be drastic, the solid part of the diet being epsom salts and glucose and the liquid part water. In milder cases, if they respond to a few days' treatment in bed, on a rigid diet, the pregnancy may be allowed to continue; but the diet should be absolutely protein free. Fruits and vegetables can be taken freely, and milk, cooked in any form, is well tolerated.

3. Pyelitis appears about the 20th week and frequently simulate appendicitis. Massive doses of alkalies with a ketogenic diet often help the patient to tide over an attack.

Diet during lactation.

During lactation, the regulation of mother's life is of the utmost importance. The diet should be of plain nutritious food. An ordinary well-balanced diet is best for producing a good supply of milk during lactation period. There are no galactagogues known to be of positive value. In cases where milk is poor or deficient in quantity, thick malt extracts, a table-spoonful three times a day may be prescribed. Where the fat is deficient, plenty of butter, cream and olive oil may be added to diet.

Diets containing milk proteins and animal proteins are better for producing milk than those containing vegetable proteins. Over-feeding never increases the quantity of milk. Cow's milk protein is the best form to increase the milk production and to protect the mother's tissue. Besides the food factor plenty of rest and daily bath, a daily walk in open air and as much outdoor life as possible are essential. Tea or coffee should be taken in moderation and anything containing vinegar, spices, mustard, or rich and complicated dishes should be avoided.

(3) Diet in Infancy and Childhood

It is seldom, if ever, necessary to have recourse to any artificial means of nourishing the newly-born infant. It is the baby's birth-right to be nursed by its own mother and breast feeding is always the best for both the mother and the child. It gives the greatest security against intestinal disturbances and infection which are the commonest troubles with the new-born. The child is more strong and vigorous as compared to those artificially fed and better adapted to surroundings. The infant should be nursed in this way for at least two months if not longer.

Diet in infancy.

When for some condition or other either with the mother or the child, breast feeding is contraindicated, the baby may be fed artificially. There is, however, no perfect substitute for breast milk. Although the results of careful artificial feeding are satisfactory, the problem is beset with difficulty. When a child is called upon to digest food of a different form and build up the body from materials of different quality, he naturally starts at a disadvantage. The younger the child, the greater is the difficulty. Before substituting any form of artificial food for mother's milk, the following points should be considered very carefully.

1. The choice of food.
2. The method of preparations.
3. The amount of food required in twenty-four hours.
4. The size of each feed.
5. The interval between each feed.

Cow's milk, goat's milk and ass's milk may be used for the purpose and of these, the cow's is the most generally used. From a comparison between the composition of cow's milk and that of breast milk it appears that cow's milk contains a large excess of protein and salts but too little sugar; the fat is in about the same proportion. In order to make it of the same proportion as the human milk, it should be diluted with water and some fat and sugar be added to it. One heaped tablespoonful of sugar of milk and half an ounce of cream, added to half a pint of cow's milk, and made up to a pint with water is, however, a good substitute.

Not all infants, even if normal and healthy, can be fed in the same way. The food should be modified as regards the quantity of each ingredient—fat, sugar and protein, in such a way, as to suit the requirements of the individual child.

ze of eds.

As regards the amount at each feed, it is customary to begin with 3 ounces; this may be raised to 4 ounces at the end of first month, 4½ during second month and so on. Approximately a child will take 1 to 2 ounces more at a feed for every month of age till 8 or 9 ounces.

Intervals between each feed may be four hours but what is the most essential is the regularity. The child should never be waked up at night for its feeds.

Various patent milk preparations are on the market but these should be avoided whenever possible. Excellent results may be obtained by using a milk composed of one part of condensed milk to two or three parts of evaporated milk when diluted with seven or more parts of water. Dried milks are now extensively used in warm climates and when packed in hermetically sealed tins they keep well. They should be kept dry free from insects and in their original containers.

It is sometimes very useful to add some additional factors to the artificial diet. Cod-liver oil should be started at three or four months in amount of 5 to 20 drops twice daily and continued till the age of eighteen months. Fruit juice should also be added at least two teaspoonfuls daily and gradually increased.

ndications for pecial modifica-ons.

Indications for special modifications are:—1. Flatulence and habitual colic are invariably due to over-feeding—the food being given very rapidly, most often at too frequent intervals. 2. Curds in stool—This is generally caused by feeding the baby on an unsuitable mixture or when the digestive power of the child is weak. The difficulty may be remedied by boiling the milk and dilution. 3. Vomiting, loss of appetite, constipation and diarrhœa are other difficulties in artificial feeding and they can always be dealt with by attaining the proportion of all the ingredients.

eeding of lder children.

A knowledge of nutrition of a growing child as regards both the amount and kind of food necessary for proper growth and maintenance of health is very essential. The points to be noted in this connection are—

Its constitution, *i.e.*, (*a*) vitamin, (*b*) salts, (*c*) proteins, (*d*) fats, (*e*) carbohydrates and (*f*) water.

The forms in which these elements are to be provided.

The relative proportions of the various elements and the total daily amount of each.

Under modern conditions we are faced with the difficulty of formulating a well-balanced dietary for children.

The ideal diet consists of fresh milk, cream, butter, cheese, curds, eggs, etc.

Cereals	.....	Wholemeal flour, wheat or rye—given in form of bread or cakes.
Vegetables	.....	Green vegetables and tubers.
Fruits	.....	Oranges, apples, plums, bananas. etc.
Sugar	.....	Pure honey.
Meat foods	.....	Lamb, chicken, rabbit, calves' liver may be given occasionally.

In the ordinary way the diet is laid down empirically and attention is given to the quality of the food, the quantity being determined in a healthy child by the appetite. The real test of the value of a diet is its clinical results.

The estimated requirement which determines the necessity of a generous supply of food throughout childhood and one relatively in excess of that required for the adult, may be quoted shortly as follows:—45 calories per pound body weight at one year, gradually decreasing to 36 calories per pound at six years and remaining at this figure until puberty.

(4) Invalid Diet in Tropics

A good supply of fresh milk must be provided in every hospital. Another important practical point is that invalid diets are often deficient in the anti-scorbutic vitamins; a standing order should be observed in all hospitals that every patient who is on an invalid diet must be given the juice of an orange daily or a corresponding dose of vitamin C in some other form. The prejudices of patients may give rise to a certain amount of difficulty. Many of them regard rice as the only suitable staple article of diet, so that a good deal of persuasion may be needed to induce them to take other food. Many Indians regard milk as unsuitable for those who have a cough, because of a traditional belief that the milk becomes converted into phlegm. Many patients also object to milk because they find that it causes diarrhœa. The explanation of this prejudice is that milk is usually regarded as a drink and is taken in addition to a full diet, with the result that the suitable quantities, either alone or as a substitute for other food, does not cause diarrhœa in the tropics more than in cold countries. Milk in the tropics is often boiled for a longtime; care must be taken to ensure that it is just brought to boiling point and then cooled. (*Tropical Medicine*—Rogers & Megaw, 1935).

6. Glucose (Dextrose)

Dextrose or glucose is one of the most important food-stuffs and in disease it is of great value. The origin of the present day dextrose therapy in medicine dates to Hippocrates. His aphorisms advise liberal quantities of honey or fruit syrups for the undernourished and the convalescent. This knowledge was handed down to the Romans and honey was introduced in infant feeding in the second century A. D. to relieve the symptomatology of "inanition fever." Grape sugar and glucose are the oldest and perhaps the most apt synonyms of dextrose. *Historical and General.*

Dextrose, was first obtained in pure form by a German Chemist in the middle of the seventeenth century. It was prepared on a commercial scale in the early part of 19th century by hydrolysing starch with dilute acids and this still forms the basis of its commercial production from Indian corn (maize). One hundred parts of water dissolve 82 parts of dextrose at 20-25°C. Dextrose is approximately three-fourths as sweet as cane sugar. *Chemistry.*

Solid dextrose is normally a mixture of two isomers. The two isomers differ only in physical properties and may be separated by careful crystallization, but this is of little practical importance, since the chemical and physiological behaviour of both forms is alike.

(1) Physiological Considerations

Dextrose is the first carbohydrate to be formed in plants and is the primary sugar from which higher carbohydrates are derived. It is the form into which all carbohydrates must be converted before utilisation by the animal body. There is something in the atomic arrangement of dextrose-molecule which makes it fundamentally important to both plant and animal kingdoms.

The presence of dextrose in the blood stream was first detected in diabetics in 1775. In 1855 it was described to be present in the normal blood. Dextrose is supplied to the body mainly in the form of sugar and starch foods which are broken down into monosaccharides before absorption into the blood stream. Maltose is reduced to dextrose by digestive enzymes; sucrose is broken down into one, molecule of fructose; lactose or milk sugar is similarly broken into dextrose and galactose. Dextrose thus constitutes by far the largest proportion of the monosaccharides absorbed into the blood stream. Dextrose itself, on ingestion, is rapidly absorbed from the small intestines mainly without any previous digestion. Each one of the monosaccharides has its own rate of absorption. About 0.8-0.85 gm of dextrose are absorbed in one hour per kilo body-weight; the absorption is independent of the concentration and the total quantity of dextrose in the intestines. The rate of dextrose absorption or its relation to body weight, however, are variable factors probably. The actual mechanism of absorption of dextrose into blood stream is complex. *Presence in animal body.* *Absorption.*

Conversion into Glycogen.

After absorption, the monosaccharides are transported by the portal vein to the liver where they are converted into dextrose through the intermediary stage of glycogen. In the liver most of the dextrose is synthesized into glycogen but some passes into the systemic circulation for distribution to and utilisation by the tissues. After ingestion of 90 to 100 gm of dextrose by normal fasting persons, approximately 70 to 80 per cent of the dextrose is stored as glycogen, and only 20 to 30 per cent is burned by the tissues for energy purposes. If very large quantities are administered, the dextrose in excess of that assimilable by the liver and tissues for glycogen synthesis or oxidation, is converted to and stored as fat.

Tolerance to dextrose.

The capacity of an individual to utilize or tolerate dextrose is the dose which may be administered without producing glycosuria. The rate of utilization of dextrose by the normal human body was found to be 0.8 to 0.9 gm per kilo body weight per hour. Continuous intravenous infusion of dextrose solution at the rate of utilization of dextrose does not produce glycosuria in normal persons. Ingestion of as much as 500 gm of dextrose at a time does not result in glycosuria. The high rate of absorption and utilization of dextrose as well as the high renal threshold render it the best tolerated sugar. Accurate determination of the ability of the body to utilize dextrose in health and disease, are now possible and dextrose tolerance tests, have been standardized. These tests are invaluable to the physician in estimating disturbances of carbohydrate metabolism. The current destrose tolerance test consists in evaluating the blood sugar and testing the urine for dextrose before and at half hourly intervals after the ingestion of 100 gm of dextrose. If a person has a high and prolonged rise in the blood sugar level, it indicates a low dextrose tolerance, and shows a typical diabetic blood sugar curve. Various factors such as preceding diet and exercise modify the dextrose tolerance test. If the subjects for the test are given a carbohydrate free diet during the several days previous to dextrose tolerance test, they likewise show a diminished tolerance; conversely administration of a high carbohydrate diet during the period preceding the test increases the tolerance of the person for dextrose. Physical exercise previous to the test may manifest itself in a hyperglycæmic dextrose tolerance curve in a normal person.

The most favourable conditions for a dextrose tolerance test include a previously unrestricted diet, and the elimination of physical and emotional disturbances before or during the test.

Recent modifications.

More recently modifications in the dextrose tolerance test have been suggested in order to eliminate the variations in the process of absorption, and some authorities advocate intravenous injection of dextrose. The one hour two dose dextrose tolerance test has been introduced. Most consistent and precise results are said to be obtained by administration of 50 gm of dextrose at half hourly interval instead of single large dose and testing the blood and urine for sugar one half hour after each dose.

Regulation of blood sugar level.

There are certain conditions which modify the rates of glycolysis and dextrose utilisation by the tissues, and the hormones are the most important factors from this point of view. The animal organism is unable to utilise dextrose without insulin. The cells of the islands of Langerhans of the pancreas are sensitive to the concentration of blood sugar. Their activity is stimulated by the presence even of a very slight excess of dextrose in the blood. As a result more insulin is produced in order to mobilise the excess dextrose which is then promptly removed from the blood stream. If for some reason excessive amounts of insulin are produced in the body, too much dextrose will be mobilised and removed from the blood stream; nervousness, hunger and fatigue then appear. If the blood sugar falls below 0.07 per cent these symptoms become accentuated until at about 0.04 per cent death takes place. Conversely, if insulin production in the body is deficient, the blood sugar will not be mobilized and utilised by the body at the normal rate and hyperglycemia will result.

Action of hormones.

Epinephrine or adrenalin and suprarenin, also play an important part in blood sugar regulation. The physiological action of epinephrine is antagonistic to that of insulin; it has a depressor action upon the utilisation of dextrose by the tissues.

The pituitary hormone resembles epinephrine in its effect upon carbohydrate metabolism. Its excess often produces hyperglycemia and diminished dextrose tolerance of the body. The exact mechanism of action is not fully known but it is believed it acts as a stimulus to the adrenal glands and does not influence the blood sugar directly.

Thyroxin, the hormone of the thyroid gland reduces the dextrose tolerance of the body. It causes the glycogen content of the liver to diminish gradually, perhaps through,

stimulation of glycolysis. As in the case of pituitrin, it has been suggested that the effect of thyroxin is indirect and takes place through the intermediary of adrenal glands.

(2) Modes of Administration

Dextrose may be introduced into the human body by mouth or per rectum and parenterally by subcutaneous, intraperitoneal, intramuscular or intravenous injection. The most natural and common route is by mouth. It is the most convenient since absolute purity and exact dosage and concentration are unnecessary. As much as 1 lb may be ingested per diem without any harmful effects. The oral administration is indicated where dextrose serves as a food and source of quick energy and where a specific effect upon the alimentary tract is desired. It may be usefully combined with fruit juices or extracts. *Oral administration.*

The efficacy of the rectal administration of dextrose is controversial despite its widespread application in clinical medicine. For rectal administration the solution should be hypotonic (2-3 per cent) or isotonic (5 per cent). Such solutions are least irritating and least likely to be expelled. The solution should be administered slowly to eliminate the danger of rectal distention. Approximately 5 ounces of 3-5 per cent dextrose solution are given to adults at six hour intervals or by slow drip method. Other substances as salt, sodium bicarbonate or alcohol may be added. *Rectal administration.*

The injection of dextrose permits the direct introduction of an immediate source of energy into the tissues or blood stream. When dextrose therapy was first developed, reactions characterised by chills and fever occured occasionally. To-day, however, reactions following the parenteral administration of dextrose are rare. The impurity of the ingredients of the infusion mixture is found to be responsible for the greatest number of reactions. The water fraction was recently shown to be the most consistent cause of reactions. The solutions should be prepared in pyrogen free water and then sterilised. All coloured, opaque or crystallised solutions should be discarded. *Parenteral medication.*

Subcutaneous.—The technique of hypodermoclysis is simple. The most suitable sites for injection are the flanks, anterior folds of the axillæ, and the upper and outer parts of the breasts. A 5 per cent dextrose solution is apparently least irritating although some clinicians have obtained satisfactory results with a 10 per cent solution. Subcutaneous administration may be painful at times, due to the pressure of an excessive rate in injection or to excessive concentrations of dextrose solutions. Hypodermoclysis is an effective substitute for the intravenous injection in the treatment of diseases of infancy and childhood, but is only infrequently resorted to in adults.

Intraperitoneal administration.—The injection of dextrose solution into the peritonium is an excellent procedure during infancy and childhood, the most suitable site being the line below the umbilicus. A hypotonic solution is indicated since those of greater concentration tend to draw body fluids into the abdominal cavity increasing the possibility of distension. The method is simple and quick and is not painful; the absorption is very rapid.

Intramuscular administration.—The lateral and anterolateral surfaces of the thighs are preferable to the buttocks; both may be used. The quantity of solution to be injected varies with each case depending upon distension of the muscles about the site of the injection. Infants usually take 20 to 40 ccm of a 10 per cent dextrose solution with ease, older children and adults up to 100 ccm at a time. Unlike hypodermoclysis the intramuscular injection is usually not painful and is applicable to adults as well as infants and children.

Intravenous administration.—The intravenous injection of dextrose solutions is also an excellent means of administering specific drugs which cannot be introduced into the body by another route. However, perfect asepsis, an assembled apparatus and skill in the technique of administration are essential for good results. Intravenous dextrose may be given either intermittently or continuously. The intermittent intravenous administration of dextrose is best performed at the veins of the elbows and ankles in adults. In infants and young children the superior longitudinal sinus and the veins in the scalp are preferred. The solution must be maintained approximately at 37°C. The intermittent method is advantageous in the treatment of conditions in which osmotic pressure relations are involved (promotion of diuresis, restoration of peripheral circulation, etc.). The continuous method, on the other hand is preferable when body fluids must be restored. In the intermittent method 300 ccm at time of a 25 per cent dextrose solution may be

given at the rate of 4 ccm per mimute. This is repeated as often as necessary, blood sugar levels and clinical condition of the patient serving as guides to the number of injection.

(3) Glucose in Therapy

General.

There are few conditions in medicine and surgery in which glucose cannot be used with considerable advantage to the patient. It should, however, be used rationally and method of its administration should be carefully considered with due regard to the patient's condition. If possible oral route should be used but where this is not feasible it should be given by hypodermoclysis, intramuscularly or intravenously as occasion demands and in proper concentrations. If necessary it should be combined with insulin so as to ensure its assimilation by the tissues. Hypertonic solutions up to 50 per cent in strength can be given intravenously with a view to remove collections of fluids from the body by diuresis. Such solutions are also used to relieve arterial hypertension and increased cerebrospinal tension. If intravenous injection have to be used, glucose used should be pure and it should be dissolved in pyrogen-free double-distilled water.

Surgical.

In Surgery administration of glucose helps in stocking the liver with glycogen, and in this way the toxic effects of the anaesthetic are minimised. Some surgeons give intravenous injections of glucose starting a day or two before the operation. Glucose administration is particularly useful in operations on the gastro-intestinal and urinary tracts and such conditions where nourishment by the mouth cannot be given.

Gastro-intestinal deficiency conditions.

Glucose (dextrose) is useful in a large number of diseased conditions. It can be given with benefit in cases of severe vomiting and diarrhoea, either by the mouth or by the parenteral route whichever is feasible. In peptic ulcer, glucose given by the mouth is considered by some to have a neutralizing effect on the acidity of the stomach contents. It is also believed to directly reduce hyper-acidity by stimulating the secretion of insulin which is said to inhibit the secretion of gastric juice. It would also provide the much needed nutrition to the muscle tissues. Glucose requires no digestion and therefore is not irritating. The motility of the stomach is said to be reduced.

In coeliac disease in children, subcutaneous injections of glucose with insulin may counteract faulty carbohydrate matabolism and thus improve the disordered fat metabolism.

In most liver diseases the glycogen contents of the organ is on the low side; administration of glucose will help in replacing the depleted stock of glycogen and in this way combat the accompanying hypoglycaemia.

In deficiency diseases there is a chronic condition of starvation of the tissues and glucose gives good results, administered if necessary, with insulin and deficient vitamins. It is particularly useful in marasmus in children and conditions of malnutrition accompanied by diarrhoea. As much as 250 gm may be given during the day.

In cardio-vascular disease especially when the heart is failing it may be life-saving by supplying much needed nourishment to the myocardium.

Allergy.

In allergic conditions there may be hypoglycaemia and consequently the glycogen content of the liver is reduced. This decreases the resistence of the body and allergic conditions set in. Glucose helps by stocking the liver with glycogen which counteracts this. When given by the mouth it may

decrease putrefaction of proteins in the gut by bacterial organisms and in this way prevent their absorption into the circulation. Lactose is preferable in such conditions.

Infectious and other serious conditions.

In infectious disease the toxins produced give rise to increase in the metabolism in the tissues. As digestion is often disturbed, the absorption of nutritive substances from the gastro-intestinal tract is inhibited. In such conditions glucose given by the mouth (it needs no digestion) is absorbed and thus prevent tissue breakdown. If it cannot be taken by the mouth adequate quantities may be given by the parenteral route. In such disease as pneumonia, smallpox, cerebrospinal fever and other serious infections it may be a life-saving factor. As much as 600 gm may be given either by the mouth or by injection (equalling about 2400 calories). In diphtheria it may be combined with serum treatment. In cholera 5 per cent glucose along with saline is useful in stimulating diuresis. In tetanus a 25 per cent solution may be combined with magnesum sulphate solution (25 per cent) and given intravenously. In cases of tuberculosis it may supply much needed nourishment to the system. There are in fact very few conditions both in medicine and surgery where it cannot be useful if given rationally.

In blackwater fever its use is the most essential part of the treatment. In fact in almost all tropical diseases glucose can be used with advantage.

CHAPTER IX

VITAMINS

VITAMIN A: SHARK LIVER OIL—VITAMIN B COMPLEX: VITAMIN B_1 (ANEURIN, THIAMIN), VITAMIN B_2 (RIBOFLAVIN, LACTOFLAVIN), NICOTINIC ACID, VITAMIN B_6 (PYRIDOXIN, ADERMIN), FOLIC ACID ETC.—VITAMIN C (ASCORBIC ACID)—VITAMIN D (CALCIFEROL)—VITAMIN E —VITAMIN K—OTHER VITAMINS.

It has been shown clearly that a diet besides containing suitable quantities of all the well recognized constituents is incapable of maintaining the body in a healthy condition unless minute quantities of substances are present which are now known as vitamins. These vitamins are necessary for normal growth to occur and normal health to be preserved. There was a tendency at first to regard them as highly unstable in character and to doubt whether they would ever be isolated or their chemical nature revealed. That outlook has profoundly altered. Many of the vitamins have been isolated in very nearly pure condition. It has been shown that they are substances of comparatively simple chemical constitution. Funk proposed the name 'vitamine' for these substances, as he thought that chemically they are of the nature of amines. The name 'vitamin' has now come to stay though it is spelt without an 'e'.

Sources.—The vitamins occur in a great variety of foods, but appear to be formed mainly in plants. They are rather unstable. Vitamins are stored in the body for short periods. Vitamins are present in most of the natural foods both animal and vegetable, particularly in glandular organs and products including milk, eggs and liver and in green vegetables and fruits and in the embryo and hylla of cereals. They occur in yeast. Meats, potatoes, flour, most oils and sugar products are relatively deficient in vitamins.

The vitamin content of some of the common classes of food-stuffs is given below:—

Vitamin A is present in most animal fats, except lard and is derived from *Carotene,* a yellow pigment in green vegetables. Shark liver oil is a rich source and so is cod liver oil.

Vitamin B or B_1, or *aneurin* is found chiefly in meat, milk and eggs and *Vitamin* B_2, or *lactoflavin* in yeast, wheat germ, whole cereals and pulses.

The anti-pellagra factor (P.P.) or *nicotinic acid* is found in liver, yeast and probably in other foods with vitamin B_2.

Vitamin C or *ascorbic acid* is found in fresh fruits, especially citrus fruit and vegetables.

Vitamin D is usually associated with Vitamin A in most animal fats.

Vitamin E like Vitamin A and D, also occurs in animal fats, but is present in some vegetable oils which do not contain the other two fat soluble vitamins.

It has been shown that vitamins combine with substances such as ferments which are concerned with metabolic processes in the cell especially in the provision of energy from carbohydrate food. Thiamin, and nicotinic acid and riboflavin are probably concerned with processes of cellular nutrition and respiration and are components of co-enzymes which are used up and need constant replacement. They are therefore present in all cells. Avitaminosis is probably due to disturbance of cellular functions due to failure of co-enzyme action and chemical phenomenon, and depend on the grade of vitamin depletion. There are, however, other factors at work also.

Deficiency of vitamins in dietary produces effects according to degree to which each vitamin is deficient and the duration of the deficiency. The clinical picture thus varies, according to the degree of deprivation, its duration and the energy output of the individual. Further in the presence of clinical syndrome, changes occur in the gastro-intestinal tract which interfere with normal assimilation of diet and furthur increase deficiencies, a vicious circle being thus created. The results are evident during war and famine conditions. The deficiencies are usually multiple and besides proper dietary, concentrated preparations of vitamins have to be given along with certain minerals as iron, calcium and copper.

1. Vitamin A

Occurrence.

Vitamin A occurs most abundantly in animal tissues, particularly in the intracellular fat of liver and glandular organs and, therefore, in butter fat, egg-yolk, fish-fat, fish-roe, fish liver, cod liver oil and A_2 in fresh water fish. It is also widely distributed in plants, especially in the green leaves. Vitamin A is apparently synthesised only by the chlorophyll of plants in the presence of light; lower plants without any chlorophyll cannot produce it. Germinating seeds do not contain it until they have produced green leaves. Animals derive their supply of vitamin A from the green plants directly or indirectly and store any excess especially in their livers and fatty tissues. The vitamin A content of foodstuffs of animal origin (milk, butter, eggs, etc.), therefore, is governed by the diet of the animal.

The original source of the fat-soluble vitamins in cod-liver oil or shark liver oil is the chlorophyll-containing diatoms, which are eaten by plankton organisms and these, in their turn, by the small fishes which form the food of the cod. Vitamin A in milk is derived from the fresh vegetables in the cow's diet and hence the milk of grass-fed animals is rich in this vitamin, but that of stall-fed animals may contain very little. In nature, vitamin A is always associated with the vitamin D.

Carotene (provitamin A).

The yellow colour of many vegetables, especially carrots, sweet potatoes, yellow corn and certain vegetable oils, is due to a lipoid-soluble substance which has been isolated in a crystalline form. This substance has been designated as *'carotene'* and it has further been identified in animal kingdom, *e.g.,* in fish, hen's eggs, butter, corpus luteum, blood, etc., being derived probably from the food. There are four types of carotene, α, β, γ and Cryptoxanthin of which α and β carotene is the most important

Carotene C_{40} H_{56} is a hydrocarbon which is widely distributed in bacteria and higher plants, usually associated with chlorophyll. The amount of absorption of carotene from different foods varies and is only about 20 per cent from raw carrots; fats and bile are necessary for its absorption. It is stored in Kupffer's cells in the liver and is converted into vitamin A there by the enzyme carotinase; its storage and conversion is altered in disease of this organ. Vitamin A is never excreted in man in health except as a breakdown product. Herbivorous animals depend entirely on carotene for their vitamin A.

Vitamin A must form part of human food; it occurs only in few articles of diet mentioned above. Carotene is however, widely distributed in vegetables and fruits. Domestic cooking does not cause great loss of this vitamin, but prolonged boiling does. The maximum requirements of vitamin A is 60 I. U. and of carotene 200 I. U. per kilo. For normal growth and freedom from clinical symptoms 20 I.U. of vitamin A and 40 I.U. of carotene daily per kilo are required; pregnant women require larger quantities. Diseases of the gastro-intestinal tract interfere with absorption and those diseases which interfere with digestion of fats, *e.g.,* sprue, hinder it considerably.

Carotene produces complete protection in animals on a vitamin A deficient diet. The two substances, carotene and vitamin A, generally but not necessarily, occur in the same food-stuffs. Carotene can be easily obtained and purified and has now been accepted as an International Standard for comparison of the vitamin A content of foods.

The International Unit (I.U.) is the "Reference Cod-Liver oil" of the U.S.P., which contains 3,000 I. U. per gm. The International Standard unit is 1 μ (0.001 mgm) and 3 to 5 units daily are sufficient to protect young rats.

Composition and properties.

Vitamin A was synthesized in 1937. It occurs in pale yellow crystals melting at 5.5 to 6°C to a viscous oil. It is soluble in oil and fat solvents and also fairly soluble in water. It is less stable than vitamin D, being destroyed by oxidation. By passing oxygen through heated cod-liver oil, vitamin A is completely destroyed. In butter, this vitamin is confined

to the portion of the fat with low melting point and is not destroyed by steam. In ghee it is destroyed at the frying temperature of 200°C.

Vitamin A has been concentrated into a fraction of the unsaponifiable lipoids of cod-liver oil. It is believed that it is a labile oxidation product of oxycholesterol.

Physiological effect.

An adequate supply of vitamin A in the diet is essential for normal growth in childhood. It is essential for the maintenance of epithelial cells in a healthy state at all ages; it is an essential group in the visual pigment rhodopsin (visual purple) which in its absence cannot be synthesized. This substance is broken down by the action of light setting free a carotinoid pigment. The subject thus loses the power of vision in dim light; this can be cured in a few hours by the administration of vitamin A. Night blindness is the first symptom of vitamin A deficiency, and if treated early, will prevent degenerative changes in epithelial tissues. As the trouble progresses, opaque spots are seen on the sclera; then there is conjunctivitis and blepharitis, and if not checked ulceration of the cornea occurs, leading, to blindness. Famine and industrial depression bring the disease to light. In extreme cases, the skin becomes thickened and epithelium of the upper respiratory tract may be similarly affected.

Recognition of deficiency.

Vitamin A deficiency can be recognized by (1) measuring dark adaptation and (2) by estimating it in the blood. This vitamin is necessary for maintaining the structure of body cells and its plentiful supply increases longevity and delays senility. The effects of deficiency are seen in all the epithelial surfaces of the body (skin and mucous membrane) and some are damaged earlier than others. Changes are not pronounced as a rule in glands, but germinal epithelium of the testes is affected. It is essential for urinary secretion and kidney function. Probably the thyroid stimulates the conversion of carotene to vitamin A. Deprivation of vitamin A causes degeneration of the nervous system and it has been suggested that probably this is the cause of lathyrism; other effects are respiratory infection and diarrhœa.

Hyper-vitaminosis

Although as much as up to 200 I. U. per kilo are sometimes given few untoward effects have been observed. High carotene level in the blood may produce decreased resistance to respiratory diseases and occasionally carotinaemia (Xanthosis cutis). In this condition the skin and conjunctiva may become yellow (may be mistaken for Jaundice) and is sometimes met with in the tropics, especially in diabetics on an incorrect diet.

Vitamin A and resistance to infection.

It is not correct to say that the administration of vitamin A reduces the liability to respiratory infection. No relation between vitamin A and the resistance of the body to infection has been demonstrated; 600 I. U. represents the maximum daily requirements in man; infants require as much as adults. The richest sources are halibut or shark liver oils, which are given in doses of a few grams. The blood content of vitamin A can be estimated and vitamin A deficiency readily detected. It is raised in conditions where storage capacity is defective resulting in carotinaemia.

Colour test for vitamin A.

When arsenic or antimony trichloride is added to an oil containing vitamin A, a blue colour is produced and the intensity of the colour is roughly proportional to the vitamin A content. This test provides a simple and rapid method for the approximate estimation of the vitamin A content of fats and oil and obviates the necessity for the biological test which requires 8-10 weeks. The absorption spectrum of the colour produced in this test has been determined.

Biological estimation of vitamin A is based on growth response of young rats. The diet given to them contains all essentials except vitamin A so that arrest and resumption of growth can be attributed with certainty to the vitamin content alone.

The following tests are also used:—

Measurement of vitamin A deficiency.

(1) By Photometer:—The eye is exposed to bright light so that visual purple is bleached. After this the light is extinguished and the patient is shown small test spots of light. The illumination of the test spots is reduced without making them invisible as the eyes become adapted to dark. The lowest illumination which after ten minutes, allows the spots to be seen is taken as the measure of dark adaptation. By this test the patients can be quickly divided into normal and sub-normal groups.

(2) By Blood examination:—For normal blood the value is 100 to 300 I.U. per 100 ccm though it tends to be low in early spring. The method employed is based on trichloride of antimony colour reaction and matching the depth of the colour of carotene against solutions of potassium bichromate or coloured glasses. The process is assisted by the photocolorimetric method.

Carotene is useless to the body unless converted to vitamin A; its estimation in the blood is, therefore, of little clinical value.

Shark Liver Oil

During recent years, a great deal of interest has centred round the production of shark-liver oil, especially on the West Coast of India. The fish liver oil is no stranger to India. About eight decades ago, India exported quite large quantities to the West, Calicut being a big centre of production. Around 1870, the price of cod-liver oil fell below that of the Malabar fish oil and the Indian Industry practically died out for want of a suitable market. Cod-liver oil from Norway and other countries began actually appearing on the Indian market in increasing quantities and the country became more and more dependent on foreign supply. The German invasion of Norway in World War II, followed by British blockade, practically cut off that country from the rest of the World. The subsequent submarine Warfare and the increased demands of the other nations kept India in short supply of cod-liver oil. Against this the demands of the civil population and the fighting forces in the country for some suitable source of Vitamin A, became quite considerable. An alternative source had to be found and under this stress, the Malabar fish liver oil industry was reborn.

The high Vitamin A content.

It was soon established that the liver oil from the shark and the saw-fish has a much higher Vitamin A potency than cod-liver oil. Occasional samples have potencies of several hundred thousand International units per gram, i.e., well over 100 times the potency of standard Norwegian cod-liver oil. The average potency is however, of a lower order, though still, distinctly higher than that of cod-liver oil. In some areas, an average of 30,000 units has been reported. In others, over 10,000 is found to be the average. Genuine samples having a potncy of between 5,000 and 6,000 units are common. A minimum potency of 10,000 units per gram has been suggested for the Indian Addendum to the British Pharmacopoeia and this may not be difficult to attain in practice. An oil of this standard will have about six times the potency of average Norwegian cod-liver oil.

Deficiency in India.

According to the latest International standard, the average daily requirement of an adult would be about 5,000 units of Vitamin A. The majority of people in India get very much less than this quantity in their food and for this the symptoms of deficiency are commonly met with among poorer classes. A large section of the population would require between 3,000 and 4,000 units daily as a supplement to make the required minimum. The supplement has to come in the form of fish liver oils, vitamin or carotenoid concentrates etc., but very few actually get them. The majority go without it, with the result that gross deficiency manifests itself in different forms. Early blindness, tuberculosis and other wasting diseases are some of the ultimate consequences of such persistent deficiency; apart from these, millions more show a sub-normal condition. In fact, it is generally recognised that there is more extensive dificiency of Vitamin A than that of any other vitamin in India.

Supposing that about 10 per cent of the population of India, is to receive a supplement of 2,500 units per day of shrk-liver oil (possessing an average potency of 10,000 units per gram), the total requirement of the country would be roughly a million gallons. The present annual production of shark-liver oil from all sources is not more than 35,000 gallons. There is evidence to show that all the oil is not derived from genuine shark or saw fish.

Supplementing with Vitamin D.

The cod-liver oil is naturally rich in Vitamin D. In that oil vitamins A & D are so proportioned that no further supplementing is needed. In the case of shark-liver oil, vitamin A is very predominant, so that further supplementing of vitamin D will be needed to render it more balanced. Fortunately, concentrated Vitamin D preparations like calciferol are easily available at relatively small cost so that it should not be difficult to supplement shark-liver oil with vitamin D at the blending centres. Considering the importance and the inter-relation between these two fat-soluble vitamins, it should be made a state policy to insist on the supplementing of shark-liver oil with Vitamin D before marketing.

During the first few years of the shark-liver oil industry, it was considered desirable and necessary to dilute the vitamin A content to the same level as in cod-liver oil. Groundnut oil was largely used for this purpose. This practice led to a number of secondary difficulties. It also facilitated the marketing of a number of spurious products. Even when used in drops, any fish-liver oil has a disagreeable smell. Several ingenious methods for taking such oils along with food have been suggested, but even then, the more fastidious people cannot miss the smell. It has been suggested therefore that the vitamin in the oil should be concentrated free from smell. Vitamin in the shark-liver oil has been concentrated by molecular distillation and chemical method in Haffkine Institute, Bombay and odourless and good tasting tablets have been prepared with chocolate base. These are absolutely odourless and quite pleasant to taste. These tablets are being now turned out in millions and are being issued to the people.

2. The Vitamin B Complex

Constituents.

This group is now shown to consist of two factors, one thermolabile and the other thermostable. It contains a number of distinct principles, B_1 or Aneurine or Thiamine, B_2 or Riboflavin, Nicotinic acid or Niacin, B_6 or rat anti-dermatitis factor or adermin.

Other allied vitamins are *Vitamin* H or Biotin which is shown to be a cyclic urea compound containing sulphur with carboxyl group. It occurs in high concentration in tumours.

Choline is present in B group and maintains normal kidney structure.

Adenylic acid (a complex of adenin, ribose and phosphoric acid) occuring in yeast is a respiratory mediator concerned with oxidation-reduction mechanism in body cells.

Pantothenic acid is a filterate factor necessary for growth in rats and an anti-dermatitis factor in chicks, but its role in man is not known. In beriberi its blood level is 20 to 50 per cent below normal.

(1) Vitamin B_1 (Aneurine, Thiamin)

Aneurine hydrochloride B.P., (thiamine hydrochloride U.S.P.) was formerly known as vitamin B_1. It is the thiazol derivative of 2-methyl-6 amino-pyrimidine, and is a sulphur containing vitamin. It is colourless freely soluble in water and may be obtained in a pure state by extraction from natural sources or by synthesis. In dry condition it is stable at 100°C. Destruction in cooking is not great unless sodium bicarbonate is added to vegetable, but pressure cooking causes rapid destruction.

Sources.

Aneurine is incapable of being synthesised by animal organism and animal tissues can store only very small quantities. In vegetables it is found in the embryo of plants, just under the husk in the grain of cereals such as wheat and rice, and also in nuts and seeds such as peas and beans. In the process of milling or polishing of wheat and rice, however, the embryo and therefore most of the aneurine, is removed, leaving mainly the starch store of the grain behind. Malt and yeast are rich sources, but alcoholic beverages prepared from malt and yeast are almost devoid of aneurine. The yeast cells being unable to synthesize aneurine, use up all the store present in malt during their multiplication.

Almost all fresh vegetables and animal foodstuffs such as milk, meat, eggs, fruit, etc., contain some aneurine and this deficiency is rarely met with except in populations existing solely on a diet of polished rice.

Absorption and storage.

Aneurine is readily absorbed and stored in the tissues, particularly in the liver, kidneys and muscles; it is abundant in heart. Man can store considerable quantities, as beri-beri does not develop till after two or three months on a diet deficient in aneurine. The explanation of the rarity of mental changes in beriberi as contrasted with the severity and frequency of peripheral neuritis, is to be found in the fact that the brain tissue retains its store of aneurine more tenaciously than other tissues. Prolonged diarrhœa interferes with the absorption of aneurine.

Subcutaneous injection of 50-500 mgm aneurine per kilo body weight increases oxygen intake by 20 per cent in guinea pigs. It produces diuresis probably of central origin. Because of great difference between its therapeutic and toxic dose, it is safe for prolonged therapeutic use. Man is unable to synthesize or store it to any appreciable extent. It is readily absorbed from the small and possibly large intestines and therefore escape absorption in digestive disturbances. In diseases associated with increased output of urine considerable quantities may be wasted out before it can be utilized.

Action.

Aneurine is essential for proper carbohydrate metabolism, and is necessary to all forms of plant and animal life. Oxidation of carbohydrates to carbon dioxide and water takes place through the intermediate stages of lactic acid and pyruvic acid. Aneurine is essential for the complete breakdown of pyruvic acid into CO_2 and water, and in aneurine deficiency, pyruvic acid accumulates in the tissues. Experimental evidence has shown that addition of aneurine to tissue preparations deprived of this vitamin, results in a greater consumption of oxygen and greater evolution of carbon dioxide. The blood and tissues of animals and men suffering from aneurine deficiency contain high quantities of pyruvates and it is probable that symptoms of the deficiency disease result from the decreased power of tissue respiration of the heart and nervous system. The degeneration of the peripheral nerves in beriberi may thus be explained.

Aneurine deficiency in non-rice eating communities.

Only the rice eating peoples suffer from beriberi. Even with the poorest diets in other peoples, beriberi does not develop. There are several conditions, however under which even well-to-do people may develop aneurine deficiency: (1) Diminished absorption of food associated with many chronic gastro-intestinal diseases, such as pyloric stenosis, chronic ulcerative colitis. (2) In chronic alcoholism, subjects may drink very large quantities of alcoholic drinks, such as beer, and may lose their appetite for fresh foods. The increased intake of carbohydrate also leads to increased demands for aneurine, thus aggravating the deficiency of the diet. A proportion of chronic alcoholics certainly do develop aneurine deficiency resulting in alcoholic heart disease and peripheral neuritis. These changes are often accompanied by mental changes (Korsakoffs syndrome) and it has been claimed that these changes are cured by nicotinic acid.

Symptoms and signs of moderate deficiency—Occuring chiefly in adults, the following symptoms are common: distaste for food, unwillingness to consume a full diet, postprandial distension, digestive disturbances, neurasthenia, lassitude, skin dysaesthesias, and calf muscle tenderness.

Symptoms and signs of severe deficiency—The following are the specific deficiencies benefitted by Thiamine (B_1) administration.

Beri-beri—severe anorexia, polyneuritis, serous effusion, subcutaneous œdema, and cardiac insufficiency.

Alcoholic neuritis—with its attendant excruciating limb pains.

Neuritis of pellagra—although niacinamide is the specific treatment for pellagra, pain, numbness, and other neurologic signs are relieved by thiamine (B_1).

Neuritis of pregnancy—the symptoms of peripheral neuritis late in pregnancy are frequently alleviated by vitamin B_1.

Deficiency induced by excessive metabolism—unless adequate supplies of thiamine are administered, high fever, an elevated basal metabolic rate (thyrotoxicosis, heavy muscular work), excessive sweating, and even intravenous glucose solution, may induce this deficiency.

Psychic changes—negativism, general mental depression.

Tests for aneurine deficiency.

Two colour tests can be used for demonstrating diminished aneurine in the blood in cases of suspected deficiency: (i) The oxidation of aneurine to thiochrome with potassium ferricyanide in an alkaline medium. Thiochrome is flourescent under ultraviolet light. (ii) *The formaldehyde azo test.* Demonstration of increased quantity of pyruvic acid in the blood is also an indication of aneurine deficiency. (iii) *Bradycardia method.* Yonug rats put on a B_1 deficient diet loose weight and heart beats come down from 500 to 550 per minute to 350. (iv) *Phycomycetes test.* Growth of the mould *P. blakesleanus* is proportional to the concentration of B_1 in the medium. It is used in the estimation of B_1 in whole blood, plasma, urine and cerebrospinal fluid.

Normal requirements.

The daily average requirement for an adult is about 1 mgm; infants require about one quarter of this amount, and the requirements in pregnancy and lactation are five times the normal adult average. In pregnancy large amounts of aneurine are required and it is believed that the polyneuritis of pregnancy is a result of aneurine deficiency.

Increased physical work, pregnancy and hyperthyroidism necessitates increased intake, as the utilization of aneurine is directly related to that of carbohydrates.

The international unit is the antineuritic activity of 3 gamma of pure B_1, *i.e.*, 300 I. U.=1 mgm. The minimum daily requirements for an adult of 70 kilo weight (11 st.) on 3,000 calories a day is approximately 300 I.U. or 1 mgm, but 500 to 700 I.U. is desirable. Infants need 50-60 I.U. (0.2 gm).

Dosage and therapeutic use.

In deficiency states 2 to 4 mgm are usually required to secure rapid improvement. However, in doubtful cases large doses, 10 to 20 mgm may be given for a week before the therapeutic test is held to be negative. Aneurine should be given to all cases of alcoholic peripheral neuritis and heart failure. It should also be tried in all cases of peripheral neuritis and myocardial weaknesses of obscure origin.

Aneurine hydrochloride is obtinable in tablets and in sterile aqueous solution in ampoules. The hypodermic route should be used for administration in cases where the deficiency is due to defective absorption as in pyloric stenoses or chronic diarrhoeas. Where there is loss

of appetite and defective tone of the gastro-intestinal tract, the parenteral route is also advisable. In other cases it can be given by the oral route.

No evidence has been brought forward to show that over-dosage produces any ill effects.

Multiple deficiency.

Diets deficient in one vitamin are often deficient in others. Test administration of one of the B group of vitamins gives more accurate information for diagnostic purposes than giving of foodstuffs rich in all members of this group. If deficiency of one member of this group is demonstrated it may be assumed that deficiency of the others either exists or is near at hand, and the proper treatment would be to provide all the vitamins in abundance.

(2) Vitamin B_2 (Riboflavin)

Vitamin B_2 (Riboflavin) is also known as lactoflavin, Vitamin G. It crystalizes in yellowish brown needles with no sharp melting point; its solubility is slight (2.5 part per thousand at 25°C). It is soluble in fat solvents and is stable in strongly acid solution and unstable in alkalies when exposed to light or irradiation with ultraviolet light. It should, therefore, be stored in amber coloured ampoules.

Occurance.

This vitamin is widely distributed in plants and animals, milk, yeast, fish roe, kidney, liver and leafy vegetables being the best source. It is heat stable and is not destroyed unless the medium is alkaline. It occurs in yellow pigment of tubercle bacillus. Its formula is 6:7 dimethyl-9 (d-1 ribityl) isoalloxazine. The Bourquin-sherman unit is equivalent to 2-2.5 microgrammes of pure riboflavin.

Riboflavin plays an important part in metabolism forming part of a complex enzyme system concerned in oxidation-reduction processes in living cells. It aids the oxidation of carbohydrates, amino acids, lactic acid and aldehydes.

Deficiency symptoms.

Symptoms and signs of moderate deficiency are vague probably including lowered general health and retarded growth. A tendency to seborrhoeic dermatitis episodes may appear. In serious ariboflavinosis, any or several of the following signs may appear; (*a*) *cheilosis,* with fissuring about the nares and mouth, or other apertures; (*b*) *qlossitis,* with purplish-red colour and flattened papillae; (c) *seborrhoeic dermatitis.* chiefly of the face and scalp; (d) *conjunctivitis,* with photophobia, dimness of vision and accomodation defects; (e) *dysphagia,* with gastric discomfort and malaise.

Most animals and men cannot synthesize this vitamin and it must be obtained from the diet. In animals kept on riboflavin free diet conjunctivitis and keratitis are produced due to defective metabolism in the cornea and lens. Vascularisation of cornea is a reliable early sign of deficiency; there is marked proliferation and enlargement of limbic plexus. The ocular lesions are cured by riboflavin therapy; photophobia and itching respond within 48 hours with doses of 5 to 15 mgm and resolution of keratitis takes place later. Acne rosacea and associated keratitis are examples of ariboflavinosis and curable with riboflavin. Cheilosis and pallor of mucosa at the angles of the mouth eventually giving rise to transverse fissure extending downwards from the angles of the mouth, is also due to ariboflavinosis.

Absorption and excretion.

Riboflavin is absorbed from the intestines and its absorption is delayed in certain gastro-intestinal diseases. If intake is increased the amount stored in the liver is increased slightly. It is excreted in urine as riboflavin.

Requirements in man.

450 units (0.9 to 1.2 mgm) daily by boys and girls under 6 and 7 years, 7 to 10 years require 540 units (1.08 to 1.32 mgm) and adults require 600 units or (1.2 to 2.5 mgm).

(3) Nicotinic Acid (Niacin)

Nicotinic acid amide was known as the pellagra preventing factor (P.P. factor) and is prepared by the oxidation of nicotine or by laboratory synthesis. It is *B-pyridine B-carboxylic acid* and its amide-nicotamide—is a compound of complex system of enzymes. Nicotinic acid is a white crystalline solid melting at 228-229°C; it is soluble in hot water and alcohol. It is one of the most stable of vitamins and is not destroyed by exposure to air, cooking, light or alkalies. It can be sterilized by autoclaving. Being an acid it forms salts. It occurs widely in most forms of animal and vegetable life and is believed to be actively concerned in the metabolic processes. It occurs in meat products, liver, eggs, fish and cereals. White bread contains smaller quantities than whole meal. Nicotinic acid is an essential group in certain enzymes, the co-dehydrogenases, which are essential

Occurance.

for the transfer of hydrogen in the oxidation processes of body tissues. It has been also shown that inhibition of the activity of these enzymes in brain tissue may be produced by narcotic drugs and it is possible that the cerebral disorders and tissue changes in pellagra may be due to lack of nicotinic acid amide, and consequent lack of co-dehydrogenases.

Estimation.

Nicotinic acid is estimated by colour test with 2:4 dinitrochlorobenzene, also with cyanogen bromide and aniline. In the blood it is estimated by growth promoting actions on various micro-organisms. Biologically it is estimated by its cure of 'black tongue' in dogs.

Nicotinic acid contents of most food varies from 1 to 100 mgm per 100 gm of material and certain crude liver extract may contain as much as 200 to 300 mgm per 100 gm.

Vasodilator action.

Toxic effects are produced by over dosage of nicotinic acid in animals, rarely in man; the amide is better tolerated. It has a vasodilator action and produces flushing of the face and neck, sensation of heat, itching and tingling, which pass off in half an hour. Throbbing headache, nausea, vomiting and transient abdominal pain, urticaria, cyanosis may occur. Nicotinic acid given intravenously produces increased flow of blood in brain in man without any change in blood pressure. In doses of 100 mgm orally it always produces slight flushing and fall of blood pressure; in doses of 30 mgm it may also do so. Slight increase in gastric and intestinal mobility may produce a transient general vasodilator effect. It is stated that nicotinic acid amide does not produce any of the minor unpleasant effects associated with administration of nicotinic acid.

Absorption and excretion.

Nicotinic acid is absorbed from the intestine and in disease of the gastro-intestinal tract this may be interfered with and lead to deficiency. It is probably stored in the liver and in pellagra the liver is damaged and liver extract is effective in this condition. In pellagra liver the hæmopoietic factor is not lost but the anti-pellagra factor is deficient. The blood level of nicotinic acid is 0.3 to 0.5 mgm per cent, the greater part being in blood corpuscles. Oral administration of 150-300 mgm of either the acid or the amide produces a transitory rise in blood level in an hour. Nicotinic acid is excreted in urine partly as such and partly as trigonellur.

Deficiency symptoms.

Symptoms and signs of moderate deficiency are the pre-pellagrous state, which is not well-defined, but mental depression, gastro-intestinal disturbances, dermatitis, and glossitis in mild form are likely to appear. The evidences of deficiency are closely similar at all ages. That of severe deficiency is manifested by the disease known as pellagra. The symptoms and signs include gastrointestinal dysfunction, exfoliative glossitis together with red-purplish appearance of the tongue, exfoliative dermatitis of the arms and legs, profound mental depression, and frequently additional signs and symptoms of riboflavin and thiamine deficiency.

Alcoholic dementia.

Alcoholic dementia and mental disturbances occurring as a result of chronic gastro-intestinal disease, may be the result of nicotinic acid deficiency often accompanied by aneurine deficiency as well. Prolonged previous treatment with bromides in such cases may delay mental improvement due to retention of bromine in the tissues, and this factor should be borne in mind when evaluating the success of treatment with nicotinic acid.

No simple specific test is so far available for nicotinic acid deficiency, but trial treatment and estimation of co-dehydrogenase in blood and urine can be carried out.

The daily requirement in man is 30 to 60 mgm; minimal requirement to prevent pellagra is 8 to 16.5 mgm (0.12 mgm per kilo) daily.

Administration.

The normal human requirement or protective dose is not known. In the treatment of pallagra the effective oral dose is about 500 mgm daily. In order to avoid unpleasant side effects it is recommended that this dose be divided into ten smaller doses of 50 mgm each. If intravenous administration is necessary the total daily dose may be reduced to 80 mgm. When marked improvement occurs the dose may be reduced to 100 mgm daily by mouth. Beside pellagra, nicotinic acid is used in stomato-glossitis, sprue and allied conditions, leukoplakia, pruritis, lupus erythematosis, eczema, psychosis due to defective nutrition, meniere's disease and sulphonamide intolerance in doses of 150 mgm.

(4) Vitamin B_6 (Pyridoxin, Adermin)

It was isolated in 1938 and it forms one fraction of the factor originally known as vitamin B_2 complex. It occurs naturally in cereals, egg yolk, liver, seeds, rice, etc., and can also be synthesized.

The chemical formula for pyridoxine is 2-methyl-3-hydroxyl-4:5 dihydroxy methyl pyridine. It is a white crystalline powder with a bitter taste and melting point 157° to 160°C.

Lack of this vitamin in the diet is the cause of "rat acrodynia," characterized by dermatitis of the paws, nose and ears. Many investigators have reported B_6 necessary for growth in chicks. This vitamin probably assists in the metabolism of unsaturated fatty acids. Recent evidence has indicated that when equal doses of pyridoxine hydrochloride and thiamine hydrochloride are administered in substantial therapeutic dosage by mouth, many cases of hyperemesis gravidarum are relieved of their excessive nausea and vomitting.

Deficiency symptoms.

Chick cleared up the confusion regarding the dermatitis syndrome of pyridoxine deficiency and showed that rats deprived of vitamin B_6 developed typical dermatitis and were cured when fed on pure vitamin B_6. It has also been shown that after treatment of typical pellagra with thiamin and nicotinic acid the symptoms of insomnia, irritability, weakness, etc., are removed by administration of pyridoxine, in 50 mgm doses intravenously. It has been used in the treatment of pseudo-hypertrophic muscular dystrophy, in Parkinsonism, arsenical peripheral neuritis and chorea. It is useful in angular stomatitis.

(5) Folic Acid

Introductory and General—The name folic acid was originally given to an almost pure chemical substance isolated from spinach; it is found in many other green leaves including grass, and in mushrooms, liver, and yeast. Folic acid will take its place among the vitamins; it has many natural sources and exists in several, probably closely related forms in the same way as vitamins. The folic acid compounds have been studied under several names, and the following are now known to be folic acid variants—vitamin M, deficiency of which causes a pellagrous syndrome of anæmia, leucopenia, diarrhoea, and mouth lesions in monkeys; vitamin B_c deficiency of which causes a nutritional anæmia in chicks; vitamins B_{10} and B_{11}, responsible for growth and feather development in chicks; and the "eluate factor" (from liver) and the "*L. casei* factor," both essential for the growth of *Lactobacillus casei* and *Streptococcus faecalis* R. The *L. casei* factor from liver has been synthesized and its constitutional formula has been established; the name "pteroyl glutamic acid" has been suggested for this folic acid compound which will so far as is known, produce all the effects of the other forms. The *L. casei* factor from yeast differs only in the number of glutamic acid molecules. Folic acid appears to be distinct from the hæmopoietic principle of the liver. It is also distinct from the primary factor which is the liver fraction active in very small amounts especially in the presence of accessary substances. (Jacobsan & Subba Raw, 1937).

Occurance.

Chemistry:—Similarities between properties and activities of *L. casei* factor and folic acid were observed by many workers and Subba Raw et al (1945) synthesised folic acid identical with the natural *L. casei* from liver. Angier and his colleagues (1946) have worked out the structure of *liver L. casei* factor or folic acid and have described two methods of preparing it. It is a compound of p-aminobenzoyl-1 (+) glutamic acid and a pteridine, 2-amino-4-hydroxy-6 methyl pteridine; the linkage is through the 6-methyl group.

The more interesting of the two methods of preparation utilises glutamic acid, p-aminobenzoyl chloride, and propionic aldehyde all of which are normal laboratory reagents and 2.4.5. triamino-6-hydroxy-pyrimidine, which should not present any special difficulty in preparation; the yield is 15 per cent.

Folic acid is not claimed to be the liver principle; it is a bright yellow crystaline substance, whereas the most active liver preparations effective in pernicious anæmia are now colourless. The results with thymine suggest that folic acid probably acts as an enzyme or co-enzyme which sets in motion the system for the production of the liver principle. Meanwhile, here is a single substance of known composition which is therapeutically active in many macrocytic anæmias.

Deficiency symptoms.

The symptomatology of moderate folic acid deficiency is not definitely known at present. It probably consists of vague non-specific malaise, loss of appetite, weakness, and moderate pallor associated with an anæmia. Moderate folic acid deficiency in children would probably be manifested by similar vague symptoms, associated with borderline anæmias.

Symptoms and signs of severe deficiency in adults are macrocytic anæmia of sprue, macrocytic anæmia of pregnancy, macrocytic anæmia accompanying pellagra, macrocytic anæmia of gastro-intestinal origin, pernicious anæmia, and in infants—macrocytic anæmia of infancy.

Experimental work:—Folic acid is widely distributed in nature and is present in any mixed diet; natural folic acid deficiency in man has not been described, though deficiences have been experimentally produced by giving specially purified diets. Rats, and probably other mammals are not dependent on food intake for their supplies of folic acid, since, as with vitamin A, they can synthesize it in their intestine. The addition to the diet of a sterilizing sulphonamide like sulphasuxidine prevents such synthesis, and this was how Nelson and Elvehjem produced the folic acid deficiency syndrome in rats. This syndnome consisted of granulocytopenia and lack of growth, and was corrected by administration of folic acid concentrates. Leucopenia, especially lack of polymorphonuclears, is the outstanding hæmatological effect of artificial folic acid deficiency in monkeys and rats. In nutritional anæmia in man, administration of folic acid not only corrects leucopenia but its effects in improving the anæmic condition are even more powerful.

Some animal experiments on folic acid deficiency have been carried out by Endicott and colleagues. They fed rats on the usual purified diet to which all vitamin B factors except folic acid had been added, together with succinyl sulphathiazole to prevent synthesis in the bowel. The bone-marrow of these animals became hypocellular, the granular leucocytes being most affected, and the erythropoietic tissues were also depleted. The rats developed a severe leucopenia, but only some showed anæmia which was not of the macrocytic type as expected, but of normocytic type. Administration of crystalline folic acid caused a rapid regeneration of the bone-marrow with a well-marked proliferation of the granular leucocytes. In the anæmic rats there was also a temporary hyperplasia of the erythropoietic tissues. These findings, though they bear little relation to the human conditions benefitted by folic acid, confirm its connection with blood-cell formation and are likely to stimulate further studies of its effect in granulopenia.

The value of synthetic folic acid in certain types of anæmias and diarrhoeas was demonstrated by Spies in a number of cases of pernicious anæmia, nutritional macrocytic anæmia, sprue and in some other miscellaneous cases. The criteria of diagnosis were carefully chosen; the cases of pernicious anæmia all had achlorhydria, macrocytic anæmia and a megaloblastic marrow; the cases of nutritional macrocytic anæmia had free HCl in the gastric juice but were otherwise similar to the pernicious cases; cases of sprue had excess fat in the stools as well. Daily doses of 100 to 200 mgm of folic acid by mouth or 20 mgm intravenously were given. There was a reticulocyte peak about the 5th to 8th day, followed by a sustained rise in red cells and haemoglobin, and the general condition was improved. Good response was also obtained in one case with alcoholic cirrhosis of the liver and in one patient with a gastric carcinoma. Cases of aplastic anæmia, and iron-deficiency anæmia, and leukæmia showed no response. Darby and colleagues have given a detailed account of 3 cases of sprue, treated with folic acid only. With dosages of 15 mgm intramascularly daily for 3 weeks, a definite transformation of a predominantly megaloblastic bone-marrow, to a normoblastic picture was obtained. The glossitis and diarrhœa abated, though the fat content of the stools remained high; the fat glucose tolerance curves returned towards normal, and the patients put on weight. One of these patients had previously received other vitamin B factor: nicotinic acid, riboflavine, pantothenic acid, and pyridoxine for five weeks without effect.

Zuelzer and Ogden in a report on a group of severe megaloblastic anæmias in infants from birth to sixteen months of age, treated some of the patients with intramuscular liver extract and the others with folic acid. The results were comparable; there was a definite reticulocyte response, a sustained rise in the red-cell count and hæmoglobin, and transformation of the megaloblastic to a normoblastic marrow. Carruthers gave folic acid to six patients with chronic diarrhœa and an iron-deficiency type of anæmia; the diarrhœa was rapidly checked, but the anæmia was unaffected.

There is thus a considerable body of evidence that folic acid is effective in the treatment of sprue and nutritional macrocytic anæmia.

Therapeutic uses and dosage—Folic acid is useful in pernicious anæmia, nutritional macrocytic anæmia, pellagra, sprue and macrocytic anæmia of pregnancy. The dosage of synthetic folic acid is being rapidly worked out. It can be given by mouth or parenterally dissolved in disodium phosphate. For pernicious anæmia the oral route is adequate; 20 mgm is given daily in the relapse phase and this dose is maintained until remission is well established, when it is reduced to 10 mg daily or less. Doan, and Davidson and Girwood, report suboptimal results with doses of 2-10 mg daily. For maintenance, Doan reports that the dose needed varies from 40 mgm a week to 20 mgm every third week; with this regime minor neurological signs improve and no case has so far developed signs of involvement of the spinal cord tracts. Good results have been reported with single large doses of 150 mgm parenterally or 400 mgm by mouth; they produce a rapid reticulocytosis but are probably unnecessarily large. In nutritional anæmias, 20 mgm daily seem adequate, and the same dose is effective in sprue; the parenteral route has some advantage in the early part of the illness. The general conclusion is that 30 mgm is an effective daily dose for these anæmias and larger doses should not be given; folic acid is not easy to prepare and supplies are unlikely to be plentiful for some time. In the refractory types larger doses may be in order, but little information is yet available; according to Doan the maximal permissible intravenous dose is 150 mgm, since larger doses may cause histamine-like vasomotor disturbances. Doses up to 400 mgm have been given by mouth without distress.

A comprehensive review of the development of knowledge about folic acid has been published by Berry and Spies, and another appears in the *UNRRA* Bulletin. The later remarks that folic acid is valuable "in the treatment of anæmia, particularly macrocytic anæmia, irrespective of the clinical classification." The evidence so far suggests that a response from folic acid can only be expected in patients whose sternal bone-marrow reveals definite megaloblastic change, and classification on these lines has, in fact, been followed by all clinical workers with folic acid. In nutritional anæmias this classification is particularly necessary, since non-megaloblastic macrocytic anæmias are common and there is no evidence that they respond to folic acid.

(6) Other Vitamins of the B Group

(1) *Vitamin* B_3.—This vitamin has been shown to be essential for the growth of pigeons. This thermolabile factor, occurs in dried yeast and wheat embryo but has not been isolated. It may be identical with pantothenic acid.

(2) *Vitamin* B_4—A specific type of paralysis in rats and chicks results from the lack of this vitamin, the existence of which however, is doubtful. This may be identical with other known factors. Recent work identifies it with the aminoacids arginine and cystine.

(3) *Vitamin* B_5—This alkali-heat-stable, water-soluble factor has been shown to be essential for the weight maintenance of pigeons. Recent work, however, indentifies this with vitamin B_6 (Pyridoxine).

(4) *Vitamin* B_7—Pigeons develop digestive disturbances in the absence of this factor. This is present in rice polishings, but has never been isolated.

(5) *Vitamin* B_8 (*Adenylic Acid*)—Adenylic Acid or Adenosine Monophosphate, a complex of adenyne, ribose, and phosphoric acid, is widely distributed in nature, in cereals, glandular tissues and yeast from which it can be extracted. It is said to be essential for the phosphorylation of glucose, adenylic acid being first converted into adenosine triphosphate, which transfers its labile phosphate to glucose. That the energy of muscular contraction is derived from the breakdown of adenocine triphosphate is supported by evidence, this reaction being catalised by calcium ions. Ruskin reported success with iron adenylate in the treatment of agranulocytosis following chemotherapy. It is stated that adenlic acid enhances the effect of vitamin B_1, in cases refractory to treatment with the latter alone. Adenylic acid inhibits bacterial growth. Spies and collaborators reported that

adenylic acid has a powerful pharmacological action. Rapid clinical improvement has also been reported by these observers in patients suffering from malnutrition, pellagra and peripheral neuritis with adenylic acid; these patients failed to respond adequatety to yeast, and large doses of vitamin B_1 and P P Factor. However, the evidence for its status as a vitamin in human nutrition is lacking.

(6) *Vitamin B_{10} and B_{11}*—These two chemically unidentified water-soluble members of the vitamin B complex are stated to be necessary for growth and proper feather development in the chick. These factors may be identical with vitamin Bc.

(7) *Vitamin B_c*—Vitamin Bc, also known as the chick anti-anæmic factor, cures a condition of dietary deficiency in pigeons in which these refuse to grow and develop an anæmia, characterised by a decrease in the percentage of hæmoglobin and red cell volume; this condition is also curable by liver extracts. This has been isolated in crystalline form from liver and yeast. Given orally it protects rats against the hypochromic anaemia induced by sulphone drugs *e.g.*, promine, promizole, diasone etc. Recent work identifies this vitamin with folic acid.

(8) *Vitamin L_1 & L_2*—Factors L_1 & L_2, claimed to be essential for the lactation in young rats, are stated to be present in beef-liver extract and yeast respectively. It has been suggested that these factors may be identical with the liver filtrate factor of Morgan & Simms or some other factor present in the "filtrate factor." The existence of these factors has not been confirmed.

(9) *Vitamin M*—This vitamin is identical with folic acid, the deficiency of which is said to produce cytopoenia as well as lowered resistance of intestinal mucosa to infection by *B. dysenteriae* in monkeys. Oral lesions resembling pellagra have also been observed consequent to lack of this vitamin in monkeys. It is present in yeast and liver extract.

(10) *Factor U*—A water-soluble growth factor of chicks, it occurs in yeast, wheat, bran and corn. This may be identical with vitamin Bc.

(11) *Rice Polish Factor*—A factor, recently discovered, it is essential for the growth and maintenance of animals receiving all other known vitamins or factors. It is present in rice-polishings and has been suggested to be complementory to vitamin B_6 in preventing rat dermatitis. This really may be a complex, as it can be replaced by a mixture of glycine and glycuronic acid or certain pentoses.

(12) *Inositol*—This factor is essential to mice, and, like choline, is a "lipotropic factor" which prevents or cures fatty infiltration of the liver. It is a normal constituents of all plant and animal tissues. The exact role of this, in animal physiology is not definitely known though it has been suggested that it may be essential, with pantothenic acid, for normal gastro-intestinal functions.

A variety of pathological conditions have been suggested to be due to lack of this vitamin, the administration of which effects cure or improvement in such conditions. These are, alopecia and 'spectacle eye' in rats as well as pruritus and atrophic gastritis in man. It is also said to inhibit tumour growth and to prevent deposition of fat in liver and other organs in man. Owing to almost universal occurance of this vitamin in animal and plant tissues, the deficiency of this vitamin is not commonly met with in man.

(13) *Choline*—Choline is essential for the metabolism of neutral fat as well as of cholesterol. It is lipotropic and prevents deposition of excess fat in the liver of rats on a diet rich in fat. The exact mechanism of this process in not known. Deficiency of choline in the diet leads to the occurance of nodular cirrhosis of liver as also haemorrhagic degeneration of kidneys in some species. It is also protective against liver damage by toxins, such as, choloroform, in rats.

However, choline deficiency is unlikely to occur in man owing to wide distribution of this factor in animal and vegitable foods; egg yolk, nerve tissues, liver and wheat germ are rich sources of this vitamin, though it is also present in green and leguminious vegetables; milk is however not a rich source and the suggestion that a diet of cows' milk and a B coli infection may be responsible for infantile cirrhosis of liver, merits consideration.

The importance of high protein diet in the treatment of hepatic lesions is suggested from recent clinical studies. Choline is essential for certain other functions in animals such as, normal nutrition of the chick and for egg production, for the prevention of perosis or slipped tendon in the birds and for the lactation and normal nutrition of rats. In addition choline is utilised in the animal organism for the formation of acetylcholine. Choline require-

ment of dog is about 35 mg/per kg. of body weight daily; that of chick is 75 mg/daily. Generally speaking the young growing animal needs more of it than the adult. Dogs made artificially diabetic have also been found to require choline.

(14) *P—Amino Benzoic Acid*—It is a vitamin and has been shown to be necessary for the normal pigmentation of the rat, it is a growth-promoting factor for the chick, as also a factor in lactation in the rat. It is widely distributed over the entire plant and animal kingdom. The claim that aminobenzoic acid is effective in the treatment of grey hair, particularly in younger middle aged persons in whom greyness was premature is not supported by clinical evidence. Some also advocate the use of p-aminobenzoic acid in the restoration of pigmentation in vitiliginous patches in depigmented areas in patients with alopecia areata.

(15) *Pantothenic Acid*—It is isolated from raw liver and possesses the property of stimulating the growth of yeast and has been identified as the "chick anti-dermatitis factor" or the "Liver filtrate factor." A deficiency of pantothenates has been found to produce in chicks a pellagra-like syndrome, affecting the eyelids, corners about the beak and adjacent skin areas,, and claws. Feathering of deficient chicks is retarded. Pantothenates cure this condition. In rats a deficiency of this factor leads to nose-bleeding, a sticky exudate on the eyelidis and depilation about the nose. The growth of pantothenate-deficient young rats is stimulated by this vitamin, and it has been found to cure early adrenal changes in such animals. Injection of large dose of riboflavin in cases of cheilosis raises the pantothenic acid content of the blood. Hence the function of Pantothenic acid is probably associated with that of riboflavin. Pantothenic acid is apparently closely allied with the cumulative effect of the vitamin B complex and has been shown to have a synergistic effect in the human system in association with riboflavin. Pantothenates have been employed in man, but precise indications have not been determined.

(16) *Biotin (Vitamin H, coenzyme R)*—Biotin is found in foodstuffs containing other members of the vitamin B complex, particularly yeast, liver, kidney, light chicken meat, eggs and peas, cocoa and cereals. Biotin is necessary for the growth of many bacterias *e.g.* staphylococcus, strains of clostridium, yeasts and fungi. It is also a growth hormone for higher plants and a growth factor for the rat and most animal. If rats are given purified diets containing sulphaguinidine or succinyl sulphatniazol, which are bacteriostatic, signs and symptoms of biotin deficiency are produced, the effect being presumably due to interference with the bacterial synthesis of biotin in the intestines.

3. Vitamin C. (Ascorbic Acid)

Vitamin C is ascorbic acid B.P. and is also known as hexuronic acid or cevitamic acid.

It condenses with aldehydes, acetone and other ketones in the form of colourless crystals melting at 192°C. When dry and protected from light it is stable even in tropics. Autoclaving at 120°C. for 20 minutes in oxygen at pH 8 results in loss of 49 per cent, solutions of vitamin C can be stabilised by addition of small amounts of fruit acid, such as tartaric or citric acid. Vitamin C is l-ascorbic acid; dehydro-ascorbic acid is as potent an anti-ascorbutic as ascorbic acid. The I.U. is 0.05 mgm of pure l-ascorbic acid and 1.0 mgm of vitamin C=20 I. U., but this standard is not now used.

It is a colourless compound, soluble in water, and possesses marked reducing properties. It is easily oxidized and can be converted back into ascorbic acid by reducing agents. It probably plays an important part in transport of hydrogen in cell metabolism. Deficiency of this substance causes scurvy.

Occurrence. Ascorbic acid occurs in all growing vegetable tissues. Green vegetables and fresh fruits (lemons, oranges and other citrus fruit, black and red currants, strawberries, cabbage, tomatoes, potatoes, onions, etc.), contain large quantities of this vitamin and smaller quantities are contained in fresh meat and milk. Unripe seeds, *e.g.*, green peas, contain ascorbic acid, which disappears when they ripen and dry, but reappear when they germinate. It is present in many fruit juices and vegetables, but the amount rapidly decreases on storage, due to the presence of an oxydase enzyme in the plant juices. Human milk contains 4 to 8 mgm per 100 ccm, cows' milk 1 to 2.6 mgm and pasteurised milk under 1 mgm. The body can store ascorbic acid, and depends for its supply on fresh vegetables. This vitamin has been definitely proved to be a protective against scurvy.

Properties. Ascorbic acid is very unstable and is destroyed on cooking or drying. It is, however, fairly stable in the rind of citrus fruits and in tomato juice. Ordinary cooking destroys most of it in vegetables and the duration of the heating is more important than the

temperature to which they are raised. Cabbage loses about 80 per cent of its ascorbic acid content by heating to 100°C for twenty minutes or by heating to 60°C for an hour. Ascorbic acid of the citrus fruit and tomato is stable even on cooking.

Scurvy.

In scurvy osteoblast and odontoblast activity is normal, and failure of connective tissue cells to form supporting tissues leads to thinning of bones and teeth. It is said that within twentyfour hours of administration of ascorbic acid improvement begins to take place. It is claimed that utilization of ascorbic acid is higher than normal during infective processes and that it may be of significance in resistance to bacterial infections. In scurvy with severe anæmia, reticulocyte crises ensue soon after administration of ascorbic acid, suggesting that it may be an essential factor in hæmopœisis. Anæmia is frequently associated with scurvy and reacts well to ascorbic acid as do other forms of nutritional anæmias. It is also essential for wound repair and is present in young granulation tissue and adjoining skin.

In bacterial infections.

There is some evidence that vitamin C has an effect on the production of anti-bodies against bacterial infection. It also possesses bactericidal and bacteriostatic properties and inactivates certain toxins, such as *B. dysenteriae, Cl. tetani* and *Cl. oedematiens.* It is also concerned with complement activity of serum.

Physiological action.

It is suggested that vitamin C is a component of a reversible oxidation-reduction system acting as a hydrogen transporter or respiratory catalyst. This vitamin is specially abundant in the corpus luteum, the adrenals, the pituitary gland and other glandular tissue. It is said to stabilise the hormones and in scurvy symptoms resemble adrenal deficiency. It may antagonize thyrotoxin.

Administration by mouth has no effect on the blood sugar, but intravenous injections lower it in normal persons. Vitamin C is essential for synthetic processes within the cell. It is absorbed by the intestines and if this is interfered with the diseased condition results. This vitamin is stored in organs and tissues with high metabolic activity (adrenals are richest). Its blood range is 0.6 to 2.5 mgm.

Excretion.

The bulk of vitamin C is excreted by the urine, small quantities in sweat and fæces. When the tissues are saturated with large doses the urinary excretion rises. Daily excretion of 13 mgm is borderline between deficient and adequate intake.

Requirements in man.

The indispensable minimum is 25 to 30 mgm per day (0.4 to 0.5 mgm per kilo); the optimum is 50 to 75 mgm daily but even larger quantities are needed during pregnancy and in acute infections. It should therefore, be regularly supplied, otherwise there is deficiency. Boys up to 15 years require 90 mgm daily and adults 30-100 mgm.

Tests.

(1) *Intradermal test.*—Depends on the power of vitamin C to decolourize the blue dye 2:6 dichlorophenolindophenol of which 0.1 ccm of a 1 in 400 dilution is injected in the anterior surface of the forearm. When the individual is saturated the skin is decolourized in less than five minutes; in unsaturated individuals 10 minutes are required.

(2) *Urinary excretion test.*—The amount of urine required to discharge the colour of a measured quantity of 2:6 dichlorophenolindophenol is determined. There are, however, substances in the urine which do this, *e. g.,* cysteine and thiosulphates. A similar test is used for its estimation in the serum.

(3) *Capillary fragility test.*—A circular area 60 mm in diameter is marked off in the antecubital fossa and a blood pressure cuff is placed at least 2.5 cm above this. Pressure is then pumped to 90 mm mercury and maintained for 15 minutes. The pressure is then reduced and petechiae are counted. The patient should not have a hot bath on the day of the test. Capillary fragility becomes pathological with blood values between 0.10 and 0.14 mgm per 100 ccm. Value above 1.4 mgm per cent is the threshold value and corresponds to vitamin C saturation.

Therapeutic uses.

In scurvy and diphtheria it is specially useful. It has also been used in pneumonia, tuberculosis, rheumatism, typhoid, malaria, dental and oral conditions, hæmorrhagic diseases, pernicious anæmia, skin diseases and eye conditions.

Most of the dietaries of the tropics are quite well supplied with anti-scorbutic substances and therefore, scurvy is seldom found in India.

The susceptibility to scurvy varies widely for different kinds of animals. Guinea pigs develop typical scurvy after 3 weeks without green food; human beings take a much longer time to develop the disease. Rats, mice, cattle and fowl appear quite unsusceptible; apparently they are able to manufacture the vitamin (in their liver).

In infancy and pregnancy, ascorbic acid deficiency may be corrected by giving ascorbic acid tablets. The richest palatable source is fresh orange juice which contains 10 mgm per 100 ccm; tomatoes contain 13 to 39 mgm per 100 ccm; apples contain little, but cabbages, cauliflower and fresh potatoes are good sources.

Probably less than 25 mgm of ascorbic acid per day is inadequate even for infants and an intake of at least 50 mgm should be aimed at in adults. In the presence of bacterial infections 100 to 200 mgm, and during pregnancy 100 mgm is probably the minimum.

Storage.

The liver of infants at birth is rich in ascorbic acid, which becomes depleted if the child is breast fed, more rapidly if fed on cows milk, and very rapidly if heated or preserved milk is given. All active tissues contain ascorbic acid. Actively growing tumours are rich in it, and its high utilization may possibly be the cause of purpura in these conditions.

Plasma should contain 1 to 2 mgm per 100 ccm and in scurvy the value falls to 0.7 mgm or lower. The urine contain at least 25 mgm in a 24 hour specimen; if less is excreted its store is badly depleted.

4. Vitamin D (Calciferol)

Vitamin D or the antirachitic or calcifying vitamin.—Several substances have antirachitic properties, the important among these are:—

Calciferol (Vitamin D_2) is manufactured artificially by 'activating' ergosterol; it does not occur naturally.

Vitamin D_3, Sterol-7-dehydrocholesterol occurs naturally and is formed in the skin by the action of the sun. Ergosterol is best irradiated in solution, but if alcohol is used, there is liability of its being over-irradiated and forming *toxisterol.*

Calciferol is a white crystalline substance which is stable at room temperature, but which loses its antirachitic properties at 18°C; it completely dissolves in oil at 80°C. Biological assay is the only method of its estimation.

In man vitamin D is derived from sunlight acting on the body. In the absence of sunlight, carbon arc or mercury vapour quartz lamp is an excellent medium for its production. Symptoms and signs of moderate deficiency are rarely detected in adults. Probably an abnormal calcium-phosphorus utilization occurs, with possible additional dental and bony changes. In infants and children, fretfulness, sweating, constipation irregular and abnormal tooth and bone development, and malaise accompanied by hypocalcaemia occur frequently. Symptoms and signs of severe deficiency in adults are manifested by osteomalacia, usually occuring in women and complicated by tetany and chronic diarrhœa. Hypocalcaemia of parathyroid tetany is another sign of vitamin D lack. The commonest result in infants and children is rickets accompained by bowed legs, craniotabes, malformation of the costochondral junctures and various constitutional manifestations including gastro-intestinal disturbances, deficient muscular development and ligamentous relaxation. Other signs include acute spasmophilia (infantile tetany), and defective dental development.

Estimation is carried out by cure of rickets test. Groups of rats are fed on a rachitogenic diet untill rickets develops. To the diet of one group a standard vitamin D preparation is added. The bone developed, stained with silver nitrate appears as a black line. In the bone ash method, groups of rats are fed for 4 to 6 weeks with various levels of standard preparations under test. They are then killed and the femoral bones are incinerated and the amount of calcification determined.

This vitamin is present in cod-liver oil in relatively large quantities and also occurs in fish oils (*e.g.,* salmon, herrings in Western countries; *hilsa,* king fish, etc., in India), animal fats, eggs, and butter; green leaves contain only small quantities. It is now known that vitamin D is produced by the exposure of ergosterol to a special wave band of ultraviolet light. It may be produced artificially by such irradiation of the isolated ergosterol or of many foods and oils which contain ergosterol. It was noted that rickets could be cured by exposure to ultra-violet light and it was soon found that exposure to sunlight also exerted a powerful curative effect on rickets. Antirachitic properties can be conferred on many foods and oils, which are not themselves antirachitic, by short exposure to ultra-violet rays. It was shown that the active principle or the provitamin D was indeed a sterol of an unsaturated and labile type, of which ergosterol is the only known representative.

Calciferol occurs in all tissues especially in the nervous system, skin and adrenals. It was originally produced from ergot and, therefore, called 'ergosterol but is now prepared almost exclusively from yeast.

5. Vitamin E

Vitamin E is known as alpha, beta or gamma tocopherol, the antisterility or anti-dystrophic vitamin, and has been synthesised; its deficiency, produces sterility in male rats, and failure in the female to carry pregnancy to term, and possibly in other mammals also. The cause of this condition is degeneration of placenta and absorption of fœtus, and in the male degeneration of seminiferous epithelium. Degeneration of peripheral nerve fibres and spinal cord has also been shown in experimental animals and that of muscle fibres resulting dystrophy. The possibility of this deficiency being the cause of habitual abortion in women has been considered and it has even been given clinical trials with doubtful results. Wheat germ and green leafy vegetables (lettuce) are the only rich sources of vitamin E.

6. Vitamin K

Vitamin K or Phylloquinone (coagulation vitamin K). It is a yellow oil occurring abundantly in the vegetable kingdom; it is ethylphytylnapthaquinone and other napthaquinone derivatives which can be synthesized to replace it. This vitamin is fat soluble, and is probably an essential group in the prothrombin molecule; its deficiency results in prolongation of the clotting time of blood. In hæmophilia, prothrombin content is normal although the clotting time is greatly prolonged. Vitamin K occurs in eggs. Bile salts are necessary for its absorption. The natural vitamin occurs in green leafy vegetables (spinach, cauliflower, cabbage, tomatoes, orange peel, etc.,) liver, egg yolk etc. Vitamin K deficiency which is detected by a lowering of the blood prothrombin level (hypoprothrombinaemia) may occur in any of the following circumstances. (1) *Inadequate supply of vitamin K;* (*a*) Nutritional deficiencies of vitamin K; (*b*) Conditioned deficiency of vitamin K as produced by sulphaguanidine, succinyl sulphathiazole etc.; (*c*) Idiopathic Hypoprothrombinaemia; (2) *Inadequate Intestinal absorption* due to lack of bile in the intestine as in jaundice and intestinal obstruction, pancreatic insufficiency, etc.; (3) *Injury to the lung;* (4) *Infections particularly of the respiratory tract;* (5) *Haemorrhage.* Indications for the therapeutic use of Vitamin K are: (a) Neonatal hæmorrhage, in which vitamin K substances are administered to the mother prior to delivery. The administration of compounds possessing Vitamin K activity will exert no effect on hæmorrhage occuring at the time of delivery, but they appear definitely to decrease the amount of hæmorrhage that may occur secondarily following delivery; (b) Obstructive jaundice,—the bleeding tendency that develops in this condition, usually prior to surgery of biliary structures, is an indication for vitamin K therapy; (c) Hæmorrhagic states associated with ulcerative colitis, sprue and coeliac disease are affected specifically by vitamin K; (d) Cirrhosis of the liver, hepatic atrophy, and ascites are non-surgical forms of hepatic diseases which are accompanied by hypoprothrombinaemia; (e) Pulmonary tuberculosis—as an aid in preventing hæmorrhage.

Menaphthone B.P. (*Menadione, vitamin K analogue*)—It is methyl-naphthaquinone derivative. It is a yellow powder and is sold under the proprietary name of kapilon and prokyavit in ampoules containing 5 mgm dissolved in 1 ccm of oil. It should be given intramuscularly to ensure absorption; oral administration is unreliable, but if given orally it should be combined with 2 to 3 gm of bile salt. The absorption of 5 mgm produces a prolongation of clotting time.

It is useful in hæmorrhagic states of the new-born. In surgical operations and cases of the obstructional jaundice of long duration where there is tendency to bleeding, one injection before operation may raise the clotting time to normal. In hæmophilia it is of no value but the administration of oestrogen, natural or synthetic, may check the bleeding. Hæmorrhagic disease of infants may be prevented by giving vitamin K to mother just before delivery in doses of 1 mgm daily.

7. Miscellaneous Vitamins

Vitamin F—It is a combination of three unsaturated fatty acids linoleic, linolenic and arachidonic acid, which are essential for the growth of yeast cells. It is not clear whether it is a pure compound or a mixture of compounds including thiamin, biotin etc., which also have this property. Linoleic and linolenic acid are present in large quantities in vegetable and seed fats though not in margarin, but the presence of arachidonic acid is doubt-

ful. Deficiencies of vitamin F produce "fat-deficiency disease" due to deficiences of the essential unsaturated fatty acids characterised by retarded and ultimately arrested growth accompanied by a raised metabolic rate, altered fat and water metabolism, changes in the skin and hair, renal degeneration and impairment of the sexual functions.

Vitamin G—It was applied to niacin amide but now it is synonymous with riboflavin.

Vitamin P—It is also called *citrin* and is said to control capillary permeability and occurs in grapes, grape juice, prunes and lemon juice. It consists of two flavone glucosides (hesperidin and eriodictol) but has not yet been synthesized.

CHAPTER X

NUTRITIONAL DISEASES

INANITION AND ITS TREATMENT: PROTEIN HYDROLYSATE, RATIONALE OF THERAPY—NUTRITIONAL OEDEMA—EPIDEMIC DROPSY: THEORIES OF CAUSATION AND CLINICAL ASPECTS, PROPHYLAXIS AND TREATMENT—BERI-BERI: THEORIES OF CAUSATION AND CLINICAL ASPECTS, INFANTILE BERI-BERI, TREATMENT AND PROPHYLAXIS—PELLAGRA: THEORIES OF CAUSATION AND CLINICAL ASPECTS, PROPHYLAXIS AND TREATMENT—RICKETS AND OSTEOMALACIA, TREATMENT—SCURVY—SPRUE: CAUSATION AND CLINICAL ASPECTS, TREATMENT—LATHYRISM.

1. Inanition and its Treatment

Introductory.—The diet of the people of India generally is very much on the low side and there are large tracts of the country where it borders on starvation. Although the modern facilities with regard to transport of food grains and cereals have done much to improve the situation, famines not infrequently occur. Famine on quite a large scale occurred in Bengal in 1943, when many millions of people died of starvation. There is a large indigent population in the towns of India who live by begging and these are the unfortunate people who suffer most. During the Bengal Famine of 1943, a large number of cases of starvation and inanition of extreme degree were brought to medical institutions for treatment. The following account of diagnosis and treatment of such cases is abstracted from the report of the Committee of Enquiry into the effects of starvation set up by Research Fund Association (I.M.G. Feb. 1944, P. 74).

"The cases admitted to hospital from the 'sick destitutes' could be divided roughly into three groups:—(*a*) those suffering from inanition due to starvation only; (*b*) those suffering from inanition due to a combination of starvation and disease, and (*c*) those showing relatively little inanition but suffering from acute disease. The methods of handling and management of these groups of cases therefore differ somewhat.

Diagnosis.—"The diagnosis of inanition is comparatively easy. The patient is usually very thin and weak and shows the characteristic mental picture of inanition, often a marked apathy. The skin is dry and cold, all the organs and tissues are shrunken, the subcutaneous fat is completely absent, the temperature is sub-normal, systolic, diastolic and pulse pressures are reduced. The eyes are shrunken and there are frequently other signs of dehydration. Often the wasting of the limbs is masked by the presence of œdema. (Care should be taken to make sure that this is not caused by nephritis). There is sometimes present a diarrhœa and colitis which may be neither bacillary nor amœbic.

"The diagnosis in patients of the second group, those showing inanition plus disease, is often more difficult. The predominating clinical picture is that of inanition, and moreover the presence of disease may be completely masked by the inanition. The patients will frequently show malarial parasites in the blood, but have no temperature and show no splenic enlargement.

"Similarly in the presence of an infection which commonly causes high fever, the temperature may be sub-normal, apparently as the result of inanition. In the same way, cases of amœbic dysentery may show little diarrhœa and stools not typical of the disease. Such patients are frequently admitted to hospital and treated for inanition, and when the general condition begins to improve they develop the fever and other symptoms which are characteristic of the infection from which they are also suffering.

"The diagnosis in patients of the third group presents no particular feature. The patients, though weak are usually not collapsed, the pulse is good though often rapid, the body temperature is frequently raised, and the patients show the typical manifestations of the diseases from which they are suffering. The common diseases found have been malaria, mostly malignant, dysentery sometimes amoebic but more often bacillary, pneumonia, nephritis, bronchitis, 'Naga Sore', tuberculosis, anæmia, urinary tract infections, etc.

(1) Treatment

"*Treatment, general.*—If the predominating clinical picture is that of disease, then the emphasis should be laid on treatment for that disease, but if, as is usual the predominating clinical picture is that of inanition, then the inanition should be treated first. Many of the patients have been exposed to cold and have little or no clothing, and a good supply of clothing, blankets and bedding is essential. The treatment of inanition and of the diseases frequently found in these 'sick destitutes' is described below:—

"*Dosage of drugs.*—In considering the treatment of these patients it should be remembered that the body-weight of most of them is abnormally low, and also that they are usually very weak. The dosage of drugs, therefore, has to be planned accordingly. In general, the ordinary standard dosage of any drug should be halved, and this principle been adopted throughout these notes.

'*Treatment, dietetic.*—Diet treatment in disease superimposed on starvation is important. A healthy person suffering from acute infection may require little diet during treatment, since he has adequate reserves. A starved person suffering from acute infection has no reserves, and the diet must be kept at the maximum possible level. Only in inanition and severe gastro-intestinal disorders should the diet be markedly restricted and then only for the minimum possible time. A highly nutritious fluid diet is of vital importance in this work.

"*Inanition.*—A considerable number of patients may be brought to hospital in a state of collapse.

"*Treatment of collapse*:—The general treatment for collapse should be adopted—rest, warmth,' warm fluids, etc., followed by a suitable diet, but in severe cases intravenous therapy is strongly indicated and special preparations of protein hydrolysates containing vitamin B complex with glucose are being made available. If this is not available, or is contra-indicated, 5 per cent glucose saline may be given.

"*Intravenous peptone glucose.*—Intravenous injections of peptone glucose are recommended for all advanced cases of starvation in a state of collapse. (Cases of group I, and sometimes of group III vide infra.) The injections help to revive the cases quickly and enable them to take suitable diet by the mouth.

"Peptone glucose for injection is supplied in screw-capped transfusion bottles. It is a mixture of 5 per cent glucose, 5 per cent protein hydrolysates and contains riboflavine, nicotinic acid and thiamin. It is of a clear port-wine colour, free from precipitate or turbidity. Every batch before issue is tested bacteriologically for sterility, immunologically for freedom from allergic reactions, and pharmacologically for absence of toxicity.

"The quantity contained in each bottle is usually 200 ccm, which represents the average dose for one injection for an adult. Bottles containing 400 ccm are also supplied.

"The bottles are best stored in the dark in a cupboard at room temperature. Storage tends to deepen the colour but this is of no consequence. Bottles showing precipitate or turbidity or giving out a foul odour on opening should be rejected. On opening the screw caps of good bottles, a noise is heard due to the rushing of air into the vacumized bottles; when the caps are found loose, it is best to reject the bottles. Once the cap is opened, the material must

be injected within 2 hours as otherwise bacterial contamination and growth will occur.

"*Selection of cases.*—Before giving injection to a patient, it is advisable to examine the urine for albumin and casts, but this is not absolutely essential. Even where no urine is available or where the patient is in an extreme state of collapse or the urine shows traces of albumin (as most cases of advanced starvation do), peptone glucose may be safely administered. But if there is general œdema, and if kidney and liver damage is suspected, it will probably be advisable to adopt other methods of treatment.

"Nutritional œdema cases may improve markedly after peptone-glucose injection.

"*Technique.*—The intravenous injections are best given with the help of Haye's pattern transfusion set. The screw cap of the bottle is removed and the cork of the transfusion set (previously washed and sterilized) fitted on to the bottle. The bottle is inverted and hung up and the clamps on the rubber tubing adjusted till the fluid comes out in drops (60 per minute) from the needle. The latter is then introduced into the vein and the fluid allowed to go in slowly. An injection of 200 ccm should take an hour to administer. Rapid administration may not be tolerated well. 400 ccm is the maximum daily dose. It may be given in one dose or preferably in two doses of 200 ccm each, one in the morning and the other in the evening. The effect is often immediate and lasts about 24 hours. The cases generally need as many as three injections on 3 consecutive days. A total of 600 to 1,000 ccm in 3 days often results in marked improvement and should be supplemented by nasal feeding (vide infra) or by hospital diet by mouth as indicated. It is essential to remember that one injection provides only about 100 to 200 calories and, therefore, as the patient improves it should be supplemented by proper diet. So far there has not been any necessity for giving injections for more than 3 days, but if required they may be continued for another 3 days with benefit.

"*Reactions.*—In none of the cases so far injected has any adverse reaction been noted. Improvement in the pulse, respiration, blood pressure and general condition usually follows the injection. In a few cases a latent malarial infection has flared up after one or more injections and prompt treatment with anti-malarial drugs has cured the disease.

"*Cleaning and sterilization.*—After using the transfusion set, it should be immediately washed thoroughly in clean water and resterilized for use. This is important, as otherwise the fluid in the set may decompose with the formation of toxic substances which if not properly washed may give rise to untoward results.

Intravenous administration of peptone glucose solution has been replaced by protein hydrolysate in the treatment of inanition. The following account of its preparation and its use in the treatment of inanition is abstracted from Krishnan et al I.M.G. April, 1944. P. 160.

(2) Protein Hydrolysates in the treatment of Inanition

"*Historical.*—Henrique and Anderson (1913) were the first to show that hydrolyzed proteins could be safely given intravenously to protein-starved animals, and that they supported life and growth, and maintained the animals in nitrogen equilibrium. Elman (1942) and Elman and Lischer (1943) found that solutions of amino-acids and peptides can be administered intravenously to man in fair amounts without any very serious reactions and that nitrogen equilibrium could be maintained by their use.

"*Methods of preparation known.*—Protein hydrolysates have been prepared by one of two ways: (1) Acid hydrolysis and (2) Enzyme hydrolysis.

Acid hydrolysis is the easier method but in this method at least one essential amino-acid (tryptophane) is destroyed by the hot acid, and has to be separately prepared and added to the hydrolyzed mixture which makes the product expensive. Acid hydrolysates are fairly satisfactory, though not absolutely safe in all cases.

Enzyme hydrolysis is not difficult and is quick and cheap, but in this method the hydrolysis is generally incomplete and the mixture may contain, in addition to amino-acids and simple peptides, higher poly-peptides which produce allergic or other reactions.

Amino-acid Mixtures—Mixtures of the ten essential amino-acids separately prepared have also been tried. While somewhat encouraging results have been obtained by some, others are of the opinion that pure amino-acid mixtures are not quite as good therapeutically as hydrolysate mixtures. Moreover, the preparations of pure amino-acid mixtures is time-consuming and expensive.

Choice of ferment and substrate—From a careful study of the methods of preparation described above, Krishnan and co-workers (1944) concluded that a properly prepared enzymic hydrolysate would be the cheapest and best product for parenteral therapy. Pancreas trypsin has two disadvantages. Firstly, the optimum temperature of its action is 37°C., and bacterial contamination is difficult to avoid during digestion. Secondly, there is always the risk of the presence of histamine or histamine-producing enzymes in pancreas trypsin.

The vegetable proteolytic enzyme papain, which is readily available in large quantities at a low cost, is the best. Its optimum temperature of action is 50°C. and the products obtained are free from histamine. While the hydrolysis produced by papain is not quite so extensive as that produced by trypsin, this does not appear to be a handicap from the immunological and therapeutic points of view. Meat proteins were selected for the substrate as its action on casein and vegetable proteins is more limited.

Preparation of hydrolysate.—The protein hydrolysate prepared in the All-India Institute of Hygiene is a papain digest of meat (vide Narayanan and Krishnan, 1941). The digestion is conducted at 50°C. for 24 hours. The extent of digestion is then tested by determination of formol titre, total solids and total nitrogen. A fractional analysis of the product is also made.. The values obtained are used as guides for standardization of the product. The undigested proteins and meta-proteins which are usually small in amount and which are likely to provoke allergic reactions are removed by repeated heat coagulation at pH7. Bacterial contamination is avoided through the use of an aseptic technique and a preservative (toluol). The final product is mixed with glucose and sodium chloride so as to give a mixture containing 5 per cent protein hydrolysate, 5 per cent glucose and 0.85 per cent sodium chloride. The mixture is then sterilized and tested before issue, biologically on cats for toxicity, bacteriologically for sterility, and immunologically for allergic reaction.

Analysis of the mixture has revealed that it contains riboflavine, nicotinic acid and thiamin, and these are of additional value in increasing the therapeutic efficiency, in promoting glucose utilization, particularly in vitamin-starved individuals.

(3) Rationale of therapy in inanition

Just as glucose is administered intravenously as a means of supplying predigested carbohydrates, so hydrolyzed proteins are injected for supplying the nitrogen needs. Where body proteins are being rapidly broken down, it is absolutely essential to administer proteins to save life. Even if sufficient glucose is given to meet the calorie needs, protein breakdown will not be completely stopped.

Protein food may be administered by mouth, but it will need to be digested and absorbed by the gastro-intestinal tract. In advanced cases of starvation, because of impairment of digestive function, this is often not possible; the only other alternative then is to administer hydrolyzed proteins parenterally. If this is done and if, along with it, glucose and vitamin-B complex are administered, the starving tissues will receive the more important elements in a form ready for utilization. Both on theoretical grounds and from the experience gained, it seems justifiable to state that administration of glucose-protein-hydrolysate in suitable amounts will not only prevent depletion of essential body and plasma proteins but will also help in the synthesis of these and in the restoration of the patients to normal condition.

Hydrolysate and serum or plasma.—Two questions may be asked: (1) why not give these hydrolysates orally, and (2) why not give serum or plasma intravenously instead of the protein hydrolysate? The answer to the first question is that protein hydrolysates can be given orally, but in patients with advanced inanition and collapse, absorption is slow and doubtful, and for quick effect parenteral administration is probably necessary. Also, after absorption from the intestine, the amino-acids first reach the liver and only later the tissues. If the liver is damaged, no synthesis will take place and there will also be delay in reaching the tissues which need them badly. Hence for the very advanced cases, intravenous injection is the method of choice. In children and in those cases where parenteral administration is difficult, oral or rectal administration of glucose protein hydrolysate may be resorted to with benefit.

To the second question the answer is that tissue proteins and serum proteins are not directly interchangeable. The latter have to be autolyzed, and the amino-acids made available for synthesis. In advanced cases of starvation where there is dehydration, the need for tissue proteins is more urgent than the need for serum proteins which may be apparently normal due to the hæmoconcentration. Thus if serum is given, its utilization will depend upon the proper functioning of the enzyme systems in the liver and tissues responsible for the splitting up of the proteins and for reconverting them into tissue proteins. The enzyme systems in inanition are not normal, and such benefit with serum may not be attained as with hydrolysates. This has been found in actual practice.

Results.—The effect of intravenous administration of glucose-protein-hydrolysate is superior to that of serum in cases of inanition. The marked and rapid improvement noted in most cases denotes that the amino-acids and peptides in the mixture reach all tissues simultaneously and are being utilized by them very rapidly. The glucose present in the mixture acts by increasing the functional efficiency of the liver. It saves protein break-down and indirectly helps in the synthesis of tissue protein from the amino-acids supplied. The vitamins present help in the metabolism of glucose.

Many thousands of patients were treated with this hydrolysate with excellent results. The hydrolysate by the intravenous route is well tolerated, and reactions are few and often mild. The only definite contra-indication is nephritis. The injections are well tolerated even in presence of pneumonia, dysentery and other infectious diseases but specific treatment for these conditions should, of course, be given.

The immediate reaction to injections is good, the pulse and general condition improve but in severe cases several injections may have to be given. In some severe cases no improvement results and the patient dies.

Dietetic treatment.—The concomitant diseases occurring in these patients are treated on usual lines. Oedema and anæmia disappear by proper diet and drug treatment.

The diet should be light and easily digestible in the beginning, consisting mainly of milk, ghol, conjee, fruit juice, etc. When he gets stronger gruel diet may be given followed by solid food.

2. Nutritional Œdema

This condition is due to long continued subsistence on low diet deficient in proteins generally and of biologically complete proteins particularly. This gives rise to changes in the concentration of plasma proteins, altered osmotic pressure of the blood and consequently to oedema which may become generalised. The condition occurs especially in war and during famine periods, hence the name "War œdema" or "Famine œdema". But considering the number of starvation or semi-starvation cases incident to war and famine cases of nutritional œdema have not been very many. Patients have been seen emaciated down to skin and bones but without œdema. It is possible that some other factor (*viz.*, liver damage) is necessary besides starvation to produce nutritional œdema.

The œdema usually develops when the diet is of low caloric value and with protein contents of less than 40 gm daily. The total proteins of the plasma are thus markedly reduced and inversion of normal albumin-globulin ratio takes place giving rise to retention of water in the tissues. Occasionally such œdema occurs even when the total plasma protein level is in the normal range. In these cases it is found that the albumin fraction is low and that the globulin fractions are increased, the osmotic pressure of the globulin fraction being much lower than that of the albumin fraction.

Clinical aspects.—As a result of low diet there is progressive loss of weight, though this is made up later by retention of water. In the early stages there is pitting œdema of the lower extremities; later this becomes generalized, and, if progressive, leads to general anasarca. Diagnosis is confirmed by the determination of total plasma proteins (normal 6.5 to 8.5 gm per 100 ccm) and albumin-globulin ratio (normal albumin 4.2 to 5.7 gm, globulin 1.3 to 3.0 gm per 100 ccm).

In the early stages the total plasma protein is unchanged, the albumin is somewhat reduced and globulin correspondingly increased. Later the total protein is reduced with inversion of the normal albumin-globulin ratio. Treatment consists in giving high protein diet (100 gm or more daily) with more vitamins. Mercurial diuretics are helpful if the œdema is marked.

3. Epidemic Dropsy

Introductory.—Rice-eating people are prone to suffer from a condition of œdema which is frequently epidemic and not connected with the usual cardio-renal causes. It was pointed out by Megaw that the condition in one extreme presents the syndrome of epidemic dropsy (fever, rash, gastro-intestinal symptoms, capillary dilatation, glaucoma, etc.) and at the other extreme the picture of chronic beri-beri with peripheral neuritis. While there may be some resemblance between the two, epidemic dropsy is now considered as a distinct and separate clinical entity.

The distinctive features are that in epidemic dropsy there is no evidence of peripheral neuritis excepting pains and aches in the muscles especially of the calves. Fever is usually present which is seldom present in beri-beri. Gastro-intestinal irritation, indigestion and diarrhœa which are very common in epidemic dropsy are not encountered in beri-beri. The erythematous rash and vascular nodules (sarcoids) are characteristic features of epidemic dropsy. Common complications such as glaucoma, abortions, hæmorrhages, etc., of epidemic dropsy are not seen in beri-beri. Sucklings are affected in beri-beri, but infantile epidemic dropsy is unknown.

Historical.—In 1876-7, during a great famine in southern India, 'beri-beri' made its appearance; its chief symptoms were swelling in the legs, palpitation, and breathlessness on exertion. In 1877 an outbreak occurred in Calcutta after the rains and continued through the cold season; it recurred in the winters of 1878 and 1879, and mainly attacked the Bengalis. A similar epidemic broke out in the island of Mauritius in November 1878, and continued till April, 1879, the disease being apparently conveyed there by the labour forces from Calcutta. Outbreaks occurred in Calcutta in 1901, in 1907-8, and 1909, a special feature being neuritis in some cases. Greig who investigated the last outbreak found that mortality was high, that it closely resembled ship beri-beri and the cause was faulty dietary. Epidemics also occurred in 1910, 1919, 1926, 1927, 1929, 1930 and the largest which occurred in 1934-35 affected not only Bengal, but the adjoining areas of Assam, Bihar, and the United Provinces. The disease was prevalent in places where parboiled rice and mustard oil enter into the dietary, the chief centres being Bengal, Assam, Bihar and Orissa, and the adjoining portions of the United Provinces. Small outbreaks have also been reported from Burma, Madras, and the Central Provinces, the disease in almost all cases was mainly confined to rice-eaters.

Seasonal and class incidence.—Although outbreaks may occur at any time during the year, the main incidence of the disease is during or after the rainy season; the epidemic tails off during the cold weather months and then disappears altogether by April. Males and females are equally affected. In Calcutta the Marwaris and Europeans usually escape because they consume little or no rice and do not use mustard oil for cooking. The disease is prevalent amongst the upper and middle classes, the poorer labouring classes often escaping. These people often cannot afford mustard oil for cooking and also they do not throw away the rice water.

(1) Aetiology

Food deficiency theory.—Epidemic dropsy was once believed to be a nutritional disorder similar to beri-beri. That it is different from beri-beri is shown by its prevalence in families in whom there cannot be any question of a diet deficient in vitamins. It never occurs in infants, whereas infantile beri-beri is well known. The characteristic cutaneous manifestations of epidemic dropsy, glaucoma and abortion are not seen in beri-beri. Vitamin therapy is effective in beri-beri, but not in epidemic dropsy.

Rice toxin theory.—In many epidemics studied rice was incriminated as the causative agent and the rice-toxin theory was formulated. It was suggested that the starch cells of rice, especially that which after parboiling and milling is stored in damp, ill-ventillated places become infected with a Gram-positive proteolytic bacterial organism. A histamine-like water soluble toxin is said to be produced as a result, and it is claimed that spores of the bacilli infect the gastro-intestinal tract. Gram-positive organisms are characteristically predominant in the stools of epidemic dropsy cases. The infected rice grains often show a central softening and opacity. The diseased rice can be spotted by putting the rice in a petrie dish and covering it up with water. The diseased grains can at once be seen and if these preponderate the rice is not fit for consumption. During an epidemic stop rice and take more bread, avoid mustard and other oils.

The points in favour of the rice hypothesis are:—(1) The disease is almost confined to rice-eaters. (2) Rapid improvement follows stopping of rice diet, and relapses are common when it is resumed. (3) The severity of the disease is held to be proportionate to the amount of rice taken. (4) Infected rice when taken to a fresh locality has started an epidemic. On the other hand there are a number of points against the rice theory:—(*i*) Rice collected from unaffected families in an extensive epidemic in Manbhum District, Bihar, was the same as that of the affected families (Chopra and Chaudhari 1936). (*ii*) In Fiji, both Indians and the natives consumed the same rice but only the Indians contracted the disease. (*iii*) In Allahabad several persons who took no rice fell victims to the disease. (*iv*) Volunteers fed on infected rice did not contract the disease. (*v*) The organisms isolated from infected rice are not pathogenic to laboratory animals.

Mustard oil theory.—Mustard oil has been suspected repeatedly for the last half century. This was incriminated mainly because Marwaris and Europeans who do not use it usually escape. Adulterants in the oil, such as fungus contaminated seeds, or admixture with cheaper oils, *e.g.,* crude linseed oil were once suspected. Against this theory, however, were the points that an outbreak was reported in a Burma Jail where no mustard oil was used and volunteers fed on adulterated mustard oil did not contract the disease. In 1926 Sarkar reported an outbreak in which several people had all the symptoms of epidemic dropsy after taking oil that had been contaminated with argemone oil from the seed of *Argemone mexicana* (Katakar or Sialkanta oil). About 10 years ago Chopra and Chaudhari incriminated a brand of oil during an outbreak in Jamshedpore. An epidemiological investigation undertaken by Lal and his associates in different areas supported the mustard oil theory. In feeding experiments on human volunteers they, in clinical collaboration with Chopra and Chaudhari, were able to produce suggestive symptoms. Feeding experiments were also carried out at the Calcutta School of Tropical Medicine on human volunteers and on animals (Chopra et al, 1939) with very suggestive results. It has been shown that argemone oil a common adulterant of mustard oil, can produce symptoms of epidemic dropsy even in small quantities. Lal and his associates (1941) place the maximum safe amount at 0.5 per cent.

Infection theory.—A specific bacterial or virus infection has been suggested from time to time but there is no direct positive evidence in support of this view although the seasonal prevalence, endemicity, uniform pathological changes and clinical features and epidemics outbreaks all point to an infection.

(2) Pathology

The 'toxin' appears to have direct specific action on smaller blood vessels in the skin, heart, muscle and other tissues. Marked dilatation of the papillary and sub-papillary blood vessels of the skin leads to increased capilliary permeability and œdema. Proliferation of small vessels and infiltration round them with endothelial cells produce vascular growth commonly called "Sarcoids".

The lesions are from pin-point to an inch or more in diameter and are not of inflammatory nature. There is often increased pigmentation of the basal layer. The heart is enlarged, the right side more than the left; the individual muscle fibres are widely separated from each other by engorged capilliaries and transudative œdema, and cardiac inefficiency results. The lungs may be congested and œdematous, hydrothorax is often present; the liver, spleen, intestines, etc., generally show dilated vessels and congestion. In the eyes, the uvea is engorged with over-production of aqueous humour which may cause glaucoma.

(3) Symptomatology

The onset may be sudden or insidious. Premonitary diarrhoea is common; there may be a sudden attack of diarrhœa with or without nausea and vomiting. The patient usually

ascribes this to some error in diet, but is surprised to find swelling in the legs when the condition is actually recognized. Initial œdema is of the solid type and the skin over it may be tense, flushed, shiny and warm. The œdema does not usually extend above the knees, but may in some cases affect the thighs, sacral region, body and upper extremities. Hyperpigmentation may occur in the skin of the hands, feet and face. 'Sarcoids' occur in some cases, and may appear even on mucous surfaces of the mouth and nose. They vary in size from a pins-head to a walnut, and begin as tiny red papules which gradually increase in size; after a varying period they dry up and disappear, leaving a pigmented area. Large 'Sarcoids' are prone to become pedunculated and infected with pyogenic organisms. Hæmorrhage is apt to occur (Chopra and Chaudhari 1935).

Clinical aspects.—Fever of a mild remittent or intermittent type, especially in the acute stage is common. Dyspnœa an early feature, is complained of before gross evidence of the cardiac damage appears. Anæmia may be present. The blood pressure is variable and the pulse rate is usually rapid and may be irregular. The condition of the heart can be classified into three groups: (*a*) *Acute or fulminating*: in which the patient has the heart affected from the very start, failure progresses and death may occur within four to seven days. These cases resemble the picture of an acute left-heart failure. This type, with a high mortality, was common in the epidemics of 1926 and 1927. (*b*) *Subacute or Chronic*: here the heart is less rapidly affected, and failure, from which recovery is usual, is a slow process. These cases resemble the picture of a combined right and left-sided failure, with slight cyanosis, jugular distension and general systemic venous congestion. This occurred in the 1932 epidemic. (c) *Formes frustes*: here the heart either wholly escapes, or is very slightly affected. This type was seen in one or two epidemics. The existence of these three distinct types cannot be explained in terms of the varying degrees of histological changes in the heart wall itself. But it is easy to understand the mechanism of these three modes of cardiac failure, by recalling the picture of a general capillary dilatation. The degree and severity of circulatory failure along with a secondary cardiac weakness are directly proportionate to the degree and extent of capillary dilatation that exist. The acute fulminating type of failure starts with a very deep and extensive capillary distension and stasis, somewhat similar to that in surgical shock or acute histamine poisoning. The blood is locked up in the capillaries, and thus is side tracked from the circulation. Consequently the veins, the heart and big arteries are relatively empty, and sudden syncope and death may result.

While cardiac involvement is the rule, its severity is not uniform. In certain epidemics, severe diarrhœa is the predominant symptom and the cardiovascular symptoms are of a mild nature. In patients with constipation, the cardiac features usually progress rapidly and become serious, often leading to a fatal end. In yet another group, mild cardiovascular symptoms are associated with a high rate of ocular complications such as glaucoma; there is as a rule only slight diarrhœa and œdema of the legs in these patients. Diarrhœa is probably produced by the toxic bases in the intestines which not only damage the capillaries and produce œdema, but also produce exfoliation of the mucous lining of the gut.

The liver is often enlarged and tender. Ascites may occur in severe cases. Cough, dyspnoea and orthopnoea are observed in some cases, and are due to the cardiac condition, œdema of lungs, or pleural effusion. Rectal congestion may cause painful piles.

Neuritis is rare and the knee jerks though they may be diminished are seldom lost. There is no impairment of sensation or motor power. General asthenia, loss of appetite, weakness and insomnia are common. Glaucoma of a primary non-inflammatory type is common; haloes are seen around lights followed by a progressive dimness of vision, tension rises high and the field of vision is gradually contracted leading to complete blindness.

Abortions are common in pregnant patients. The blood picture usually shows anæmia, generally of the macrocytic ortho-chromic type.

Course.—The disease may run an acute or a chronic course. In acute cases, cardiac failure which is present from the onset, rapidly progresses and death may follow in a few days. The sub-acute or chronic cases recover in a few weeks or may hang fire for months (asthenic type), the duration averaging about six weeks. The patient is usually incapacitated for regular work for a long time. Palpitation, breathlessness on exertion, precordial pain and general weakness usually persist for a long time. The patient may lose his sight if the symptoms of glaucoma are overlooked.

Prognosis.—Prognosis depends on, the severity of the disease and complications, and as a rule is favourable. The average mortality is between 2 and 8 per cent.

(4) Prophylaxis and Treatment

According to our present state of knowledge prophylaxis should aim at avoidance of mustard oil contaminated with argemone oil for which proper public health measures should be adopted. Admixture of mustard seeds with argemone seeds during harvesting is to be guarded and every sample of oil tested for presence of argemone oil before consumption. Simple tests:—(1) Nitric acid, (2) Cupric acid, (3) Ferric chloride.

(5) Treatment

During an epidemic, exclusion of rice and mustard oil from the diet is advisable if possible, or else prohibition of parboiled rice and use of pure mustard oil are recommended. A careful watch should be kept over the cook and servants during an outbreak. Affected cases should be as far as possible isolated.

All acute cases should be strictly confined to bed until convalescence is well advanced. The intestines should be cleared by an initial purgation and fluids should be given freely to eliminate the toxins. It is not advisable to check diarrhœa especially in the initial stages. If it is very severe and exhausting, kaolin or bismuth preparations may be prescribed. Intestinal antiseptics are advocated, but are useless.

Rice should be completely eliminated from the diet and bread or chappatis given instead. If possible the patient should be removed from the endemic area. It is advisable to exclude mustard oil from the diet altogether, but if this is impossible, pure mustard oil obtained from jails should be used. If there are some gastro-intestinal symptoms milk or its preparations such as lime-whey and butter-milk, with fruit juice and glucose should be given at first, and then other articles may be added gradually as the condition improves. Otherwise the diet should be well balanced as far as possible.

Thiamine hydrochloride (Vit. B_1) in large doses should be given in severe cases, in view of the great success of this drug in the similar condition of heart-failure in beri-beri.

For the cardio-capilliary crises complete rest in bed; adrenaline in doses of 3 to 5 minims of a 1 in 1,000 solution or ephedrine (action more lasting) intramuscularly; or preferably a tincture of ephedra vulgaris 20 to 30 minims 2 to 3 times a day, may be given. Ephedra vulgaris contains both ephedrine and pseudo-ephedrine, the latter alkaloid being particularly abundant in the Indian variety. Ephedrine acts on the vasomotor nerve endings and its action is much more sustained than that of adrenaline. Pseudo-ephedrine has a weak vaso-motor stimulant action but is direct stimulant to the myocardium. The combined action of the two alkaloids is responsible for the effectiveness of this drug in epidemic dropsy. It is advantageous to give 10 grains of calcium lactate with each dose. If much œdema is present theobromine and sodium salicylate (diuretin) may be prescribed in 10 grain-doses with the tincture of ephedra with or without mercurial diuretics.

Drugs of the digitalis group, strophanthin, and euphyllin are of little value in treating the cardiac symptoms. Glucose and stimulants, such as cardiazol coramin or injection of camphor in oil have more effect in such cases. Blood letting is helpful in congestive cases, and as much as 500 ccm or more may be removed. Injections of atropine sulphate, dry cupping, and inhalation of oxygen, help to combat œdema of the lungs. Ascites or hydrothorax may require paracentesis.

Small bleeding sarcoids can be successfully dealt with by carbon dioxide snow, and large pedunculated ones are preferably removed under local anaesthesia.

For the treatment of glaucoma as long as the visual fields do not show any change there is no need for immediate operation, but as soon as defects in the visual fields become evident, operation must not be delayed.

Convalescence.—Patients should be given general tonics to combat the anæmia and general asthenia, and to expedite convalescence. Heavy exercise should be avoided for a long time, and the eyes should be periodically examined. Rice should be restored into the diet very gradually and with caution.

4. Beri-Beri

The term beri-beri is applied to a syndrome characterised by multiple peripheral neuritis and in severe cases by congestive cardiac failure, which occurs mainly in tropical and subtropical climates either endemically or in form of epidemics. It is a deficiency disease due to lack of vitamin B (thiamine), and other vitamins also.

Historical and general.—Beri-beri was known in China many centuries ago. In Japan the disease has long been known under the name of 'Kakke' meaning weakness of the legs. The name beri-beri is probably derived from a Malayan word *biribi* meaning a jerky gait. Malcolmson (1835) pointed out that both the dropsical and paralytic manifestations often occured in the same individual in India, and that they might appear simultaneously or might succeed one another. The neuritis of beri-beri has been shown to be of the same type as alcoholic, aresnical or diphtheritic neuritis.

Beri-beri was largely spread in the past in oriental countries where polished rice was a staple article of diet. It is thus seen in the tropical and sub-tropical climates of the Eastern hemisphers, the largest number of cases having being reported from China. In India it is encountered mostly in the north-east coastal area. It has been prevalent even in cold countries such as Labrador, Newfoundland, Iceland etc., among people living mainly on white flour.

Beri-beri occurs more commonly in men and among women during pregnancy and lactation, being less common in children. It may occur in the breast-fed infants of mothers with beri-beri and infantile beri-beri has a high mortality rate. Most of the cases occur during or after the hot or damp season, the disease disappearing shortly after the new rice crop has come in.

(1) Aetiology

There are a number of theories regarding the causation of beri-beri: (1) toxic theory, (2) toxi-infection theory, (3) diet-deficiency theory and (4) combined toxic and deficiency theory, but it is generally accepted to-day that the essential ætiological factor is a deficiency, absolute or relative, of vitamin B_1. This may be due to absolute deficiency of the vitamin in the diet, to an increase of the normal requirements as in pregnancy, lactation, thyrotoxicosis and febrile diseases; to faulty absorption from the intestine as in chronic digestive disorders; to faulty storage and utilization as in liver diseases, or to interference with normal biosynthesis of the vitamin in the gut. Some hold that deficiency of vitamin B_1 is not the whole story of the ætiology of beri-beri and probably there is some other factor. For instance the combined toxic and deficiency theory assumes that in some samples of rice and occasionally also in other articles of diet, there is a poison which causes a multiple neuritis, and that vitamin B_1 is an antidote to this poison. The interaction of dietetic poisons and the antidotal action of vitamin B_1 would satisfactorily account for all the puzzling variations in the symtoms of beri-beri. Serious defect in vitamins might result in the small doses of poison present in many samples of rice producing chronic neuritic forms of the disease, corresponding to the experimental polyneuritis of birds which have been fed on polished rice. On the other hand in cases in which the poisons were present in large amounts, the normal amounts of vitamin B_1 would be insufficient to prevent them from causing grave and acute symptoms. Between these extremes would come all the many intermediate forms of the disease, caused by various combinations of the doses of the poison and the antidote. The dramatic curative effect of massive doses of thiamine in severe cases in which the patient is overwhelmed by the poisons, would be fully explained.

(2) Pathology

The heart and the nervous system are affected. The cardiac changes are hypertrophy and dilatation, the striking microscopic feature being intercellular œdema. Fragmentation, fatty and hydroscopic degeneration of the cells may be found. Degenerative lesions without inflammatory changes occur in the nervous system. The nerves of the legs are usually first affected, mainly the sciatic nerve and its branches, but the lesions may occur in the medula, spinal cord (especially posterior columns and anterior and posterior nerve roots), spinal ganglia and autonomic nervous system. The vagi and the phrenics are frequently affected. The extent of changes depends on the severity of the disease. With the disturbance in nerve supply there is secondary atrophy of muscles. Marked disturbance of the mechanism which controls water balance in the tissues, occurs in some acute cases.

(3) Symptomatology

The symptoms of the disease are referrable to (*i*) lesion of the nervous system, (*ii*) cardiac changes, and (*iii*) secondary effects of œdema.

The disease has two distinguishable stages:—

(1) A period of alimentary disturbances during which there is a feeling of heaviness in the epigastrium sometimes accompanied by nausea and vomiting; and (2) a period of polyneuritis, during which there is palpitation, shortness of breath, cyanosis and weakness. Dry beri-beri, wet beri-beri and atypical beri-beri have been described which, however are not distinct, but merge into each other. The prominent clinical types distinguished are described below:—

The chronic dry type.—This type starts insidiously with slight gastro-intestinal disturbances, wasting and weakness of muscles, especially the ones mostly used, viz., extensor muscles of the thighs. The knee jerks are often exaggerated at first but are later lost. Tachycardia is present early and palpitations and shortness of breath are usual. The course of the disease is chronic, and at any time swelling may occur so that the case presents a picture of dropsical or wet beri-beri.

Peripheral neuritis.—All or some of the symptoms due to the peripheral neuritis, *e.g.*, muscle tenderness, muscular wasting and weakness, nerve tenderness and varying degrees of impairment of sensation, paræsthesiae are present. Deep sensibility of the Achillis tendon is lost. Ataxia, finally flaccid paralysis, foot drop and wrist drop may develop. In some cases cloudiness of vision or photophobia may occur.

In severe cases cardiac failure occurs, affecting chiefly the right auricle and ventricle. Præcordial pain is common.

In mild cases the disease lasts for a few weeks and subsides gradually. In severe and prolonged attacks, convalescence is slow and the patient remains weak for months or even years. The course of a case depends very much on the treatment given. With early diagnosis and prompt treatment, the disease can be arrested within a few days, but if allowed to progress unchecked, the nerves become seriously damaged and restoration of function is slow and may never be complete.

Wet beri-beri.—In this form there is a gradual onset of œdema, which may be generalised, with breathlesness on exertion. Signs of neuropathy are often not prominent and the œdema masks the muscular atrophy. There may be enlargement of the heart, marked jugular pulsation and systolic murmur. The blood pressure falls; an injection of adrenaline further lowers the diastolic pressure. The electro-cardiogram shows right axis deviation, alteration of T waves and prolongation of Q-T. interval.

Acute cardiac type.—The attack may begin with cardiac symptoms but usually the heart fails after the disease has been present in a mild or moderate form for several weeks. In this type of the disease intoxication predominates, though vitamin deficiency also plays a part. There is intracellular œdema of the heart muscle leading to poor contractility. The onset of cardiac symptoms is usually abrupt with severe præcordial pain, palpitation, rapid feeble pulse enlargement of the heart and liver, and rapidly developing dropsy with ascites and hydrothorax. The blood pressure falls and neck veins stand out. Death may occur within a few days.

Atypical beri-beri.—This is beri-beri modified by other nutritive disorders such as pellagra, scurvy or ship beri-beri or nutritional oedema. Sub-clinical beri-beri is also not uncommon and presents symptoms, such as weakness, anæmia, vague pains, mental depression and tachycardia resulting in a syndrome resembling neurasthenia

Infantile Beri-beri.—Infantile beri-beri first described by Hirota (1898) is encountered mainly in the Philippines and Japan, but isolated cases probably occur wherever beri-beri is prevalent. The disease occurs in breast-fed infants, whose mothers are suffering from frank or latent beri-beri (toxin in mother's milk suggested as cause). The first warning is that urinary secretion is diminished. Fretfulness, anorexia, low fever, wasting, pallor, vomiting, and diarrhœa or constipation are usual. The child cries constantly and meningismus, aphonia and convulsions may occur. Oedema and cardiac hypertrophy are nearly always in evidence, and sudden attacks of dyspnœa, which are often fatal, may occur. Pathological changes are identical with those occurring in wet beri-beri.

In the treatment of such cases, the infants should be taken off the mothers breast, and fed by a healthy wet nurse or on a suitable artificial diet rich in all the vitamins. Injections of vitamin B_1 should be given, and these have been reported to give excellent results. Extract of rice polish has given good results.

The mothers should be properly fed during pregnancy and lactation. When a mother's diet is deficient, rice polish extract should be given to the child daily till six months' old.

Prognosis.—Mortality may be as high as 60 to 70 per cent, but even without treatment usually below 30 per cent. The chronic type may leave muscle weakness or flaccid paralysis due to degeneration of nerve cells. Recovery in adults is slow and muscle weakness and neuritis may persist for months. With early and adequate treatment the disease can usually be arrested within a few days especially in the infantile type.

One attack predisposes to others, and persons who have once suffered, should be advised to avoid a rice diet for prolonged periods.

(4) Treatment

The two principal requirements, in the treatment of beri-beri, which must always be kept in mind, are the prohibition of rice and any other food, such as edible oil which is likely to be toxic, and the provision of a diet rich in proteins and all the vitamins. Meat, eggs, milk and leafy vegetables are given freely. Fresh milk and orange juice are given when gastro-intestinal symptoms are present.

Vitamin B_1 (thiamine chloride) should be given in doses of 10 to 20 mgm parenterally once or twice daily. In the presence of cardiac failure, intramuscular and intravenous injections of 20 to 50 mgm are advised daily till the heart and circulation become normal; often this may result in dramatic improvement. If pure thiamine hydrochloride is not available the cheaper concentrated extracts of rice polishings may be used with advantage in most cases. Excellent results were obtained in the Philippines from the administration of *tikitiki,* (alcoholic extract of rice polishings) in doses of three ounces daily. Brewers yeast in doses of six ounces daily or marmite (2 oz) may be given.

In cases of cardiac dilatation all appropriate measures for management of acute congestive failure should be adopted. Complete rest in bed should be enforced, and blood-letting may prove a life saving measure. Distention of the veins of the neck and cardiac distress are the indications for this measure. Extensive plural effusions should be relieved by tapping. Mercurial diuretics may have to be resorted to for increased diuresis.

Alcohol and arsenic are especially harmful and should be avoided.

In chronic type of disease, besides rest and nourishing diet, massage and passive movements should be carried out. Postural treatment with or without splints may be necessary to prevent deformities.

Unaffected members of families or institutions in which outbreaks have occurred, should be dealt with in the same manner as those who have been attacked.

Prophylactic measures.—The general principles of treatment apply equally to prophylaxis. Under-milled rice should be used and rice should be stored in a dry, cool and well-ventilated storehouse. The period of storage should be as short as possible. Stocks of rice suspected of causing beri-beri should not be used for human consumption. Wetted rice should be thoroughly dried as soon as possible before storage. Edible oils used for cooking should be regarded as possible sources of intoxication.

The diets should be wholesome and rich in vitamins. Eggs, meat and vegetable should form part of the diet, unfortunately beri-beri is a disease of poverty. Rice diets should be supplemented by articles of diet which are more nutritious. Soya beans and other leguminous seeds are considered to have a great protective value against beri-beri.

Test for under-milled rice.—Place rice in a small dish and cover it with Gram's iodine solution. The exposed starch turns black with iodine. If the rice is completely decorticated the entire grain turns black; if it is undermilled it is only partly stained.

In chemical test the index proposed is 1.77 per cent phosphorous pentoxide, plus fat or any rice not having less than 0.62 per cent phosphorous pentoxide. Such rice does not produce polyneuritis in pigeons.

5. Pellagra (also known as Psilosis Pigmentosa, Alpine Scurvy)

Pellagra (rough skin) is an endemic disease characterised by dermatitis and pigmentation of the skin especially of the exposed parts, gastro-intestinal disturbances and often certain mental symptoms which may be followed by true psychosis. It was first described by Casal (1725) and was known to the spainiards as 'mal de rosa'.

Geographical distribution.—Pellagra is largely confined to maize eaters being particularly prevalent in the Balkan States, Turkey, Greece, Spain, Portugal, Lower Egypt, Mexico, Brazil, Argentina, Jamaica and the Southern States of the U.S.A. Sporadic cases occur in India and the disease is frequently seen in maize-eating areas in the Kashmir valley. Pellagra attacks chiefly the poorer labouring classes especially in warm climates. It has a definite seasonal incidence, occurring predominantly in the winter of warm countries and in the spring and early summer of the temperate zones.

(1) Aetiology

Goldberger (1914) considered it a deficiency disease and he produced a condition known as 'black tongue' in dogs (which is analogous to pellagra in man) by feeding them on a pellagra producing diet. Black tongue could be cured by yeast and subsequently pellagra in man was also found to be cured by its use. The factor responsible for cure in the yeast was termed by him as the P.P. factor. Elvehjem (1937) found that the factor in yeast responsible for cure of black tongue was nicotinic acid. Later cases of human pellagra were successfully treated with nicotinic acid (niacin) or nicotinic acid amide (niacin amide). All the symptoms even the mental symptoms disappeared whether it was given by mouth or parenterally. Such cured cases, however, developed symptoms of riboflavin deficiency or thiamin deficiency or both which could be treated by administration of these substances. Vitamin B_6 and pantothenic acid also controlled other symptoms. Pellagra is thus a deficiency disease due chiefly to lack of nicotinic acid (niacin) but to some extent to deficiency of other fractions of vitamin B complex. Such articles as milk, meat, eggs, etc., which are protective are rich in nicotinic acid.

Other theories regarding the causation of pellagra are as follows:—

The maize intoxication theory.—The distribution of the disease corresponds closely with that of maize, and there is evidence that maize which has been damaged during storage is especially liable to cause the disease. Explosive out-breaks often occur. The occurrence of the skin lesions on exposed parts of the body in pellagra led to the idea that maize might contain a photo-sensitizing substance analogous to that contained in buckwheat.

Chick (1933) advanced a view which is perhaps a combination of several factors. She postulated that pellagra may be caused by a toxic substance derived from the maize diet which can be corrected by sufficient quantity of protein or perhaps by sufficient vitamin B which is present in these proteins.

Amino acid deficiency theory.—The association of pellagra with maize consumption has given considerable support to the theory that the disease might be caused by lack of proteins of high biological value. It is suggested that in pellagra certain amino acids were deficient in the diet consumed by the patients. The protein of maize flour, zein for instance, is deficient in certain amino acids, such as tryptophane and lysine.

Infection theory.—No satisfactory evidence has been adduced to show that pellagra can in any way be considered to be directly an infectious disease.

(2) Pathology

Dermatitis may or may not be present. The skin first becomes erythematous and this is followed by exfoliative dermatitis, atrophic changes and pigmentation of the affected area. There is marked stomatitis and glossitis at first and later the mucous membrane of the small and large intestine becomes inflammed and ulcerated right down to the rectum. The skeletal muscles are wasted and degenerated as is also the cardiac musculature. There is degeneration of the neurons of the whole of the nervous system and this is especially marked in the tracts of Gall and Burdach of the spinal cord. The direct pyramidal tract also shows some degeneration and scattered areas from which nerve fibres have disappeared. There is pigmentation and degeneration of the cells of anterior and posterior horns. Mental changes are said to be due to leptomeningitis, cerebral atrophy and increaseed ventricular fluid but they disappear with nicotinic acid treatment.

Symptomatology.—The disease has a wide range of severity, varying from the mild and clinically almost unrecognisable cases to the severe and rapidly fatal types. The general course of pellagra is chronic with well marked exacerbations and remissions. In general, gastro-intestinal changes occur during the early stages of the disease, and are followed later by symptoms due to degenerative changes in the central nervous system.

In areas where pellagra is prevalent many persons exhibit symptoms of poor appetite, indigestion, low intelligence, soreness of the lips and tongue, and general debility. These are undoubtedly cases of mild subclinical pellagra (prepellagra), and a number of such people develop symptoms of frank pellagra at a later stage.

Mild type.—After a few weeks or months of the above described mild symptoms, some of the affected persons become gradually worse. The tongue assumes a red and glazed appearance, the mouth becomes sore, and patches resembling sunburn appear on the dorsum of the hands, along the radial border also, some time on the back of the arms, neck, cheeks or nose.

Alternate diarrhœa and constipation are often present. Scalding sensation in the mouth (may extend to æsophagus), redness of mucosa, salivation, loss of appetite and achlorhydria are present. Nausea and vomiting may occur. As the symptoms become intense, dermatitis appears. It must be remembered that in a large number of cases skin eruption may not appear or may be very mild. In experimental cases eruption appeared 3 to 5 months after deficient diet. Patients, however, improve but relapse in the spring.

Severe type.—In severe cases diarrhoea becomes intractable, emaciation is marked, dermatitis more pronounced and extensive and definite mental changes are observed. Dermatitis when it appears is characterised by being symmetrically distributed. In most instances the lesions are confined to parts exposed to sun, viz., the dorsum of hands, wrists, forearms and feet, front of legs, face and neck, but parts liable to friction, viz., the perineum, axillae, beneath the breasts and over bony prominences, may also be affected. After desquamation a reddened shining skin is left which looks atrophied. At first there is erythema resembling sunburn. This may be followed by vesiculation and bulla-formation; the skin becomes roughened, thickened and pigmented and is sharply demarcated from the normal skin. Desquamation occurs later, beginning from about the centre of a patch. Sore mouth and scarlet glossitis are usually present. It has been shown that exposure to sun-shine of persons subsisting on pellagra-producing diet, produces erythema. When pellagrins have been treated with niacin, sun-shine produces no such effect.

The nervous symptoms are vague resembling neurasthenia at first but may develop into psychosis in long standing cases. Insomnia is frequent, there is marked depression which may indicate insanity and may end in dementia. Sensory disturbances may take

place. Burning of the feet appears before eruptions appear (symptoms of thiamine deficiency). There may be evidence of organic nervous lesion, viz., peripheral neuritis (producing pain in limbs, formication, and subacute degeneration of the spinal cord producing increased knee jerks, abnormal gait.

The blood is normal in mild cases, but in severe cases macrocytic and hypochromic anæmia is present; pernicious anæmia may rarely occur. Usual red cell count is 3 to 3.5 millions with 50 to 70 per cent hæmoglobin; leucopoenia is always present.

Complications.—In a long standing and debilitating disease, such as pellagra there is always possibility of association with other diseases. Dysentery, amœbic or bacillary, may become implanted on a diseased mucous membrane; hookworm, tuberculosis or syphilis may occur in pellagra and pernicious type of anæmia is not uncommon. Field of vision may be contracted and visual acuity decreased.

(3) Diagnosis

Though in established cases when eruptions have developed, there is no difficulty, in early cases diagnosis is not easy. It may be mistaken for sprue but here the characteristic stool and tendency to macrocytic type of anæmia in the latter disease differentiate it. Dermatitis venanata and chronic eczema may be mistaken for skin eruptions, but sharply defined edges, manner of their development and tendency to recurrence every year are helpful diagnostic features. A test for ether soluble pigments in urine may solve the difficulty. The test is performed as follows:—To 10 ccm of urine in a separating funnel 0.2 ccm of glacial acetic acid is added (to make pH 4); 15 to 20 ccm of ether are then added, the whole is well shaken and allowed to stand. After separation the lower aqueous layer is drained off and the ether is washed with 10 ccm of distilled water. To the washed ether extract add 3 ccm of 25 per cent HCl; the mixture is shaken in a test tube. In positive cases a pink or purple colour develops when HCl is added.

(4) Prophylaxis and Treatment

Public health measures in connection with a well-balanced diet are essential. The preventive action of nicotinic acid and riboflavin has not been worked out.

Even comparatively mild cases of pellagra should be prescribed adequate rest in addition to an improved diet. Severe cases require complete rest in bed and adequate nursing. Skin lesions require to be protected from sunlight and special care should be devoted to the prevention of bed sores and contractures.

Diet.—The diet should be rich in proteins, vitamins and with a high caloric value (3,000 to 4,000 calories). Fresh milk, fresh green vegetables, fruits and fruit juices, eggs, liver, kidney and lean meats are particularly rich in all the constituents of the vitamin B complex and should be advised according to availability and the scruples of the patient. Crude liver extracts by mouth, and brewers yeast have been widely used as sources of vitamins in pellagra and help to relieve most of the symptoms; 75 to 100 gm of powdered yeast should be taken daily, mixed either with milk, tomato juice or warm water and salt.

Drugs.—Nicotinic acid or nicotinic acid amide is best administered orally either in tablet form or in capsules, in doses of 50 to 100 mgm several times a day according to the severity of the case. About 500 mgm daily are usually given but in severe cases doses of 1,000 mgm daily have been advised; up to 2,000 mgm daily in divided doses, have been used without undesirable effects.

Nicotinic acid should be given daily for seven days and after the symptoms are relieved 100 mgm may be given daily as a maintenance dose for a few days. After that if the patient is put on a proper diet the improvement is maintained. In Pellagra nicotinic acid content of blood is 0.31 mgm per cent and on treatment rises to 0.55 mgm per cent. Nicotinic acid amide is a normal body constituent and its use is less likely to lead to unpleasant effects such as vasodilatation, diarrhœa, cerebral or cardiac symptoms. A single dose of 200 mgm

intravenously may produce dramatic effects. Disoriented patients with rambling speech who are unable to swallow become rational in 2 or 3 days.

In cases with reduced visual acuity and concentric constriction of fields of vision, response is rapid, vision returning to normal in a week. Such doses may produce vasodilatation of the face and upper half of the trunk, itching and burning sensation of the skin, diarrhoea, vomiting, or abdominal cramps. The drug should, therefore, be administered in small and frequent divided doses, such as 50 mgm every hour, or 100 mgm every two hours.

It must be remembered, that the very poor diet of pellagrins, besides producing the characteristic symptoms of pellagra, very frequently also produces other clinical or subclinical vitamin deficiency states. Thus nutritional cardiopathy and peripheral neuropathy do not respond to nicotinic acid therapy, and require treatment with thiamine chloride. Angular stomatitis and cheilitis which are sometime present, respond to treatment with riboflavin in doses of about 5 to 10 mgm daily by mouth. The severe cases of dermatitis which do not respond to nicotinic acid alone, may also yield to riboflavin therapy.

A mixture containing 25 per cent brewers yeast in 67 per cent peanut butter and 8 per cent peanut oil provides a cheap and palatable food that has been found to prevent pellagra and riboflavin deficiencies when given as a supplement for even comparatively poor diets. Two ounces of this preparation should be taken daily.

The most striking effects of treatment are observed in the improvement effected in the tongue and mouth symptoms. The effect is also observed in the psychology of the patient and in the processes of digestion and assimilation; mental symptoms rapidly improve with nicotinic acid in maximum doses and coramine.

Pyrazine carboxylic acid compounds and quinolinic acid have therapeutic action resembling nicotinic acid on the mouth and tongue symptoms.

Dilute hydrochloric acid is given for the achlorhydria after meals. A soothing ointment may be applied to the skin lesions. If there is evidence of neuritis vitamin B_1 injections are given.

(5) Prognosis

Before nicotinic acid treatment was introduced, the death rate was high (30 to 60 per cent). With the present treatment, if instituted early, there should be little or no mortality but taking all sorts of cases it does not exceed 2 or 3 per cent. It is essential that after treatment the patient should be put on proper dietary, otherwise there is bound to be recurrence. The cases seen by the author in Kashmir are generally of a mild type.

6. Rickets

Rickets is a deficiency disease occurring in infants from a disordered calcium and phosphorus metabolism, caused by an insufficiency of vitamin D in the diet, or to lack of exposure to sunlight. It is characteristically seen during the rapid growth period in infancy. It responds readily to treatment either with adequate amounts of vitamin D or to exposure to ultraviolet rays, provided of course that the diet is not grossly deficient in calcium and phosphorus.

(1) Aetiology

Vitamin D can be synthesized from ergosterol by the skin tissues by photosynthesis under the influence of ultraviolet rays with (wave lengths of 2,600 to 3,150 angstrom

units). It probably acts by helping the absorption of calcium and phosphorus from the intestines, and thus indirectly regulates the calcification of bone. Inorganic serum phosphorus, normally present in the amount of 5 mgm per 100 ccm of blood is reduced below 3 mgm but the serum calcium may remain normal. In coeliac disease the failure of fat absorption leads not only to deficient absorption of the fat soluble vitamin D, but also to excessive loss of calcium, which escapes in combination with the fatty acids in the stools. A special clinical type of rickets, coeliac rickets, thus frequently manifests itself.

Rickets is essentially a disease of northern climates, and is comparatively rarely met with in tropical climates, because of the abundance of sunshine. Its prevalence in India however, is often under-estimated, because though sunshine is plentiful, the diets are often very poor and minor degrees do occur amongst the children.

Osteomalacia, or adult rickets is also a calcium-phosphorous deficiency disease accompanied by deficient calcification of all bony tissues. It is, however, fairly commonly met with in the tropics, and is endemic in parts of northern India, China and Japan. In Kashmir, the disease is specially prevalent, on account of the lack of sunshine during the winter months, combined with particularly poor diets. The disease is seen essentially in women of the child-bearing age, during pregnancy and lactation, and tends to increase in severity with each successive pregnancy. Women who are kept in *purdah* are particularly prone to be affected. Lack of calcium and phosphorous and of vitamin D, in poor dietaries, aggravated by the excessive mineral demands on the body during pregnancy and lactation, are the main factors responsible.

(2) Pathology

The characteristic changes in rickets are osteoporosis and consequent softening of bones which become flexible. This results in craniotabes, deformities of the limbs, spine and thorax. There is also defective ossification of the epiphysial cartilages, and this results in the epiphysial enlargements of long bones and the beading of the ribs. Microscopically, the epiphysial cartiliages are very irregular, and the normal zones between cartilage and bone are not clearly demarcated. The bone cortex is thin and trabeculae are greatly reduced in number or may be absent. The shafts of the long bones and also the frontal and parietal bones of the skull show, besides the general osteoporosis an increased deposition of soft spongy bone below the very vascular periostium. This produces the characteristic bossing of bones in rickets. Microscopically there is deficient calcification; the osteoclasts occur in normal numbers while osteoblasts are very numerous.

The parathyroid glands are frequently enlarged, probably due to an increased output of parathormone, in an effort to maintain the falling serum calcium level by mobilising calcium from the bones. This mechanism accentuates the osteoporosis of rickets, but tends to prevent tetany by maintaining an adequate serum calcium level. However, where the bones have been excessively depleted of their calcium, tetany is seen as a complication both in rickets and in osteomalacia.

Clinical picture.—Enlargement of the osteochondral junctions produces the characteristic beading of the ribs: the so-called "rickety rosary." The chest is frequently deformed either by a deep horizontal groove called "Harrisons sulcus" on one or both sides of the chest below the nipples; or by a vertical depression on either side of the thorax. Repeated respiratory infections are particularly common in rickets and result in exaggeration of the thoracic deformities. The osteoporosis also affects the spinal vertebrae and children are liable to develop kyphosis or scoliosis. This further accentuates the thoracic deformity and also causes apparent stunting of stature.

The anterior fontanelle in infants frequently remains wide open long after its normal time of closure, and bossing of the frontal and perietal bones is commonly seen. In young infants with severe rickets there may be extreme thinning or 'craniotabes' of the occipital and perietal bones.

The softened long bones develop curvatures and may even fracture spontaneously. Epiphysial enlargements are commonly seen in wrists and ankles and in severe cases also in the fingers. The X-ray appearances are characteristic and are most often seen at the distal epiphysis of the ulna and femur as fuzzy, irregular concave junctions. Cupping may be seen at the distal ends of long bones. The pelvis may be flattened, or the public arches may be pushed in to produce a 'tri-radiate' pelvis. Dentition is usually delayed and the teeth may show defective calcification.

The general musculature in rickets is typically hypotonic and on this account, affected infants are usually fat and flabby, and are late in sitting up, crawling or walking. They

are prone to develop the characteristic 'pot belly'. Tetany is likely to occur in severe cases when the serum calcium falls below 6 mgm per 100 ccm. Nutritional microcytic hypochromic anæmia is common, and infants tend to suffer from frequent gastro-intestinal disorders. The liver and spleen are usually palpable.

In osteomalacia, patients generally complain of pain and aching in the bones, either local or generalized. Weakness is usually marked, and severe tetany commonly occurs. There is an exacerbation of symptoms during pregnancy and lactation, but in intervals between pregnancies, the disease is quiescent. Gross and progressive skeletal deformities, especially of the pelvis, thorax, spine and long bones may result. The pelvic deformities may make labour difficult, or may even make partutrition impossible.

(3) Diagnosis

Diagnosis can be made on clinical, radiological and biochemical grounds. In early cases where bony changes are not marked, X-rays will be helpful (generalised osteoporosis and vertebrae show biconcave deformity). Estimation of serum calcium and phosphorous (which are low) and serum phosphatase (which is much increased) give indication of its presence. In case of osteomalacia, deformities of lower limbs, the thorax and the spine give indication of endemic areas. Differential diagnosis from other osteoporotic diseases, *e.g.,* hyperparathyroidism is made by blood examination, the serum calcium in the latter condition is high, the phosphorous is low and phosphatase above normal levels.

(4) Prophylaxis and Treatment

Rickets can be prevented in infants by proper feeding, regular exposure of the body to sunlight (ultra-violet rays) or addition of vitamin D to the diet, from an early age. In countries where adequate sunshine is lacking, vitamin D (in form of cod-liver oil or as halibut-liver oil or calceferol) should be given to all infants between the ages of 3 months and 2 years. Concentrates are preferable to cod-liver oil if there is fat intolerance as in coeliac disease. In tropical climates the causal factor is usually a lack of calcium in the mothers diet. Thus during pregnancy specially during later months, mother's diet should be supplemented by adequate amounts of milk along with vitamin D and calcium.

Curative treatment is based on similar lines. Where the disease is active, daily doses of 2 to 3 thousand units of vitamin D rapidly effect a cure. Exposure of the infant for a few hours to sunlight daily is necessary. If not possible ultra-violet rays exposure may be given. It is started with two minutes daily, with the lamp at a distance of three feet from the body, alternately to the back and front. The duration of exposure is gradually extended to about 15 to 30 minutes. Bony deformities may require proper treatment, such as splintage or even osteotomy.

Treatment of osteomalacia can only prevent further bony deformities, and is based essentially on giving a well balanced diet high in calcium and phosphorous content and supplementing this with calcium salts and 10,000 to 50,000 units of vitamin D daily. Cod-liver oil or some proprietary preparations are the forms in which it is usually given.

Prognosis.—This is good as regards life though the infants are likely to get secondary infections which may prove fatal. Deformities gradually become less marked with growth of the child.

Renal Rickets.—This is a rare condition of defective calcification of bones associated with chronic renal disease. One form resembles rickets and the other is due to hyperplasia of parathyroids.

7. Scurvy

Scurvy is a deficiency disease caused by inadequate intake of vitamin C in the diet. It is characterised by anæmia, great debility, tendency to hæmorrhages

and softening and fragility of bones. It appears that advanced liver disease may also produce scurvy by interfering with the utilization of vitamin C. The disease is rarely seen in tropical countries, except in labour camps in uninhabited regions, where men are fed for long periods mainly on cereals without reasonable amount of fresh vegetables and fruits.

Vitamin C is easily synthesized and the synthetic product which is called ascorbic acid or cevitamic acid, is much cheaper than the natural product. Vitamin C plays a vital part in the maintenance of the intercellular substance of the body connective tissues, and thus particularly helps to maintain the formation of bones, teeth, cartillage and fibrous tissue in a healthy state. It has been demonstrated that a vitamin C deficient diet causes a marked reduction of the plasma vitamin C content, and a significant reduction in the amounts daily excreted in the urine. A daily urinary excretion of 13 mgm is the lower limit of normal, and that an output of 40 mgm implies that the patient is saturated with vitamin C. The normal range of vitamin C in the plasma in fasting individuals varies from a minimum of about 0.6 mgm to a maximum of 1.5 to 2 mgm per 100 ccm. It is lower in patients with gastric ulcer (0.14 to 0. 59 mgm). The bodily requirements of vitamin C run parallel to the rates of growth and metabolism; thus infants and growing children require proportionately larger amounts than adults, and the body requires considerably more during fever, infection or hyperthyroidism.

Saturation test.—If a healthy adult on diet with fruit takes a large dose of ascorbic acid (500 to 1,000 mgm) on successive days, he excretes in his urine nearly the whole of it in the first 24 hours and the whole of the second dose in next 24 hours. Patients with scurvy and those on deficient diet do not start to excrete even half the daily dose until more than 2,000 mgm have been ingested. The tissue probably take up ascorbic acid till saturation point is reached, while this is taking place only small quantities (8 to 12 mgm) are excreted daily in the urine. An intradermal test has been devised to determine whether the patients' diet is deficient in ascorbic acid. 0.01 ccm of a sterile solution containing 2 mgm of indophenol in 4.0 ccm of distilled water is given intradermally. If time taken for the colour to fade is more than 10 minutes, the diet is probably deficient.

Scurvy occurred in old days among sailors who lived on salt meat and biscuits for prolonged periods during sea voyages. Besides deficiency of vitamin C there was probably deficiency of vitamin A, thiamine and possibly other vitamins also.

(1) Pathology

The basic lesion in scurvy is degeneration of the intercellular cement substance or collagen of the connective tissues. Damage to endothelial cells of the capillaries produce the characteristic hæmorrhages of scurvy. The bones and teeth are softened, and sub-periostial hæmorrhages (especially of femur and tibia) are characteristic, particularly in children.

The skin is pale and dotted with hæmorrhages varying in size from pin point to large ecchymoses; the lower limbs and in infants the inner aspects of the thighs are common sites. The mucous membrane of the gastro-intestinal tract is congested and ulceration may occur in the stomach and intestines; profuse and fatal hæmorrhages may take place. The heart is hypertrophied and dilated and sudden deaths may occur on exertion. Anasarca is present, liver and kidneys are congested. Fractures commonly occur and are slow to unite and form large callus. Separation of epiphyses and beading of ribs commonly occur. In experimental animals fatty marrow of the long bones is replaced by red hæmatogenous marrow. The blood shows anæmia with normoblasts and macroblasts in large numbers. Administration of ascorbic acid rapidly produces reticulocytosis, increases red cells and hæmoglobin.

(2) Clinical aspects

Experiments show that in man depletion period of ascorbic acid from the body on a deficient diet before typical symptoms of scurvy appear is 4 to 7 months. Before this there is a period of subclinical scurvy in which such symptoms as debility, diminished resistance to infection, imperfect healing of wounds, etc., are observed. This is called the sub-scurvy stage and in this condition the patient is in an unsaturated state.

In childhood if ascorbic acid is completely absent from diet, symptoms of scurvy appear in 4 to 6 weeks; if small quantities are present in diet it will take longer. The onset of scurvy is insidious in children; the skin becomes sallow and mucous membrane

pale. The child lies in the bed quietly with hips and knees half flexed; movement give rise to pain; tenderness is due to hæmorrhage in muscles or under the periosteum or epiphysial area. Fever rarely above 101° may be present.

(3) Symptomatology

In adults early symptoms are loss of appetite, weakness, breathlessness on exertion, dull aching pains in legs and feet, stiffness of knees, lethargy and dizziness. Later, peticheal hæmorrhages may appear especially on the legs or large ecchymoses may be seen on any part of the body. Diarrhœa is an early symptom and dysentery may be associated with it. Swellings, caused by extravasation of blood into muscles, may appear and such muscles are very sensitive and may ache continuously. The affected limbs resent movement; and the so called false paralysis are seen especially in infants and children. Hæmorrhages in the retina, brain, spinal cord, intestines or any other organ, may produce a variety of lesions and symptoms.

Changes in the gums present early and one of the most characteristic features of the disease. At first fungus elevations or *'scurvy buds'* appear between the teeth, and these gradually extend till the whole gums may be generally swollen, spongy, and bleed on the slightest touch. These swellings may later break down and ulcerate, and the teeth may loosen and fall out; the patient cannot masticate his food. The breath is foul and offensive.

Anæmia is common, and is caused by blood loss and by interference with the activity of the bone marrow. The complexion is muddy and there is increased pigmentation of the skin.

(4) Diagnosis

In typical cases the condition of the gums, teeth, peticheal haemorrhages and ecchymoses together with the dietetic history, should make the diagnosis evident. In milder cases and in subclinical cases estimations of the blood ascorbic acid levels and of excretion in the urine may be carried out. The capilliary resistance test is also valuable and is performed by putting the cuff of a sphygmomanometer on the arm and inflating it to a pressure mid-way between the systolic and diastolic blood pressure of the patient. It is maintained at this level for fifteen minutes and then released. The number of petichæ on the forearm lying within a circle 1.5 cm in diameter, placed 4 cm below the bend of the elbow are counted; any number over 15 is considered as indicating capilliary damage.

Purpura and subcutaneous hæmorrhage due to leukæmia or thrombocytopenia are differentiated by making a leucocyte count and estimation of platelets and determining the bleeding time.

Complications.—Pneumonia is a frequent complication which may be fatal. Infectious diseases, such as dysentery, malaria, typhoid may occur.

(5) Treatment

The bodily daily requirement of vitamin C is about 25 mgm, and diets containing at least this minimal amount, would be sufficient to prevent scurvy. All the symptoms of scurvy usually respond rapidly to treatment with an adequate diet which should be designed to supply at least 300 mgm of vitamin C. This amount can be supplied by about 600 gm (1½ pounds) of fresh oranges. Raw meat juice contains large amounts of vitamin C but on account of risk of tapeworm infestation, this should in practice never be used. Synthetic ascorbic acid may also be given in dosage of 300 to 500 mgm daily by mouth, in severe cases 1,000 mgm or more may be given per day. In acute cases parenteral administration would be preferable. Where long-standing disease of the intestines is present, absorption is likely to be adversely affected; under these circumstances vitamin C present in the diet or in lemon or orange juice, is said to be better absorbed than pure ascorbic acid.

Experimental work on guinea-pigs has shown that these animals may develop gastric or duodenal ulcers when scurvy has been induced in them. It is not postulated that the scorbutic state in human beings predisposes to peptic ulcera-

tion, but it is, however, very likely that the healing of such ulcers may be hampered or delayed by a vitamin C deficiency. It appears reasonable to suggest that all cases of peptic ulceration should be given adequate amounts of vitamin C. What applies to peptic ulcers, applies to wounds and ulcers in the body generally, as healing processes are essentially based on fibroblast activity. Some eminent authorities suggest that administration of vitamin should be made a routine in the treatment of all cases of gastric and duodenal ulcers. They recommend that 1,000 mgm of ascorbic acid should be given on 3 consecutive days to each new patient to saturate the body, and thereafter 25 mgm should be given daily preferably in the form of fruit juice.

The body requirements are also increased during infections and fevers and fruit juices should be given in all illnesses.

(6) Prognosis

Prognosis is good if the diet is changed and the patient is put on proper treatment; all cases recover. Otherwise it is bad. Susceptibility to scurvy varies and all persons on the same deficient diet do not develop scurvy at the same time. This is probably due to the fact that some hold ascorbic acid in the tissues better than others.

(7) Prophylaxis

This depends on the fact that a person should get at least 50 mgm of ascorbic acid daily in his diet. This amount is supplied by 100 gm of fresh oranges, lemons or grape fruit. Bottled lime juice is not much good.

8. Sprue (also known as Psilosis, Ceylone Sore mouth)

(1) Introductory and General

Sprue is a chronic, afebrile diarrhœal disease caused by functional impairment of the absorptive power of the small intestines, resulting in the deficient absorption of fats, vitamins and other essential substances in the food. The prominent symptoms are the passage of large, frothy, white stools, flatulence, progressive emaciation, soreness of the tongue, and a progressive anæmia of the pernicious type. The disease chiefly affects Europeans resident in hot damp tropical countries, though recently a number of cases have been reported in people who have never been to the tropics. The writer has seen cases of sprue among the inhabitants of northern India. During World War II Elder (1944) and Leishman (1945) observed a mysterious malady which afflicted Indian troops in the north eastern zone to which the name 'marasmic syndrome' was given and which was indistinguishable from sprue. The season of occurrance of cases was March to September with its peak in June. The soldiers who suffered were mostly those living under good conditions. Manson Bahr (1941) obtained history of dysentery in 40 per cent of the cases, but in these cases it was met with only in 9 per cent of cases. In most of the cases the syndrome was fully established within two months of diarrhœa starting, loss of weight was the outstanding feature.

Digestion in sprue is usually normal, but there is deficient absorption of fatty acids and glucose, and it is these unabsorbed constituents which cause the typical bulky, gaseous, acid stools. Absorption of vitamins, hæmatinic factor, and calcium salts is interfered with and may produce a variety of deficiency symptoms. The disease is more common in females than males; it generally occurs in middle age but cases occur even in children. The onset usually occurs during the rainy season.

(2) Aetiology

The essential cause of sprue is still not understood. The disease is not caused by deficient diet. Scott (1923) considered that calcium and parathyroid deficiency produced

this disease, but it has been shown that a calcium deficiency is the result and not the cause of the disease.

A number of writers have strongly supported the infection theory, but the apyrexial course of the disease, absence of pathological changes in the gut, and the failure to demonstrate a specific infective agent, do not support such a view, inspite of the fact that it is said to run in families and there are 'sprue houses'. Vedder (1940) considered that sprue develops only in certain predisposed individuals, and that anterior pituitary dysfunction is intimately connected with the faulty intestinal absorption. Hill diarrhoea and sprue according to some have a common aetiology and it is believed that a substantial proportion of sprue cases may commence as simple hill diarrhœa. Complete recovery occurs in sprue with intensive liver extract therapy; it is, therefore, considered that no permanent lesion of the intestine exists; the failure of absorption is probably due to functional intestinal changes. It has been suggested that the basic defects, viz., loss of ability to absorb fatty acids, glycerol and glucose, is due to failure of phosphorylation and the loss of phosphorous due to failure of phospholipid formation. Napier (1943) considers that sprue is caused by an inborn error of metabolism which normally remains latent but becomes patent when the organism is subjected to certain strains and stresses, especially those associated with tropical residence. The nature of disease is far more that of a metabolic disorder than an infection. This error of metabolism leads to primarily a failure of absorption in the small intestines, of fats, vitamins and minerals and to a less extent of carbohydrates and produces all the symptoms of this disease.

(3) Pathology

No characteristic pathological changes are found. All the tissues, such as muscle are wasted and all the internal organs especially heart, liver, spleen are atrophied. In advanced stages the bone marrow shows the characteristic hyperplastic changes found in microcytic anæmia; later the marrow may become aplastic and show little or no active hæmopoietic tissue. The intestines show thinning of the mucosa, disappearance of the valvulae conniventes and thinning of the muscular coats. Others have found the intestines to be morphologically normal (in autopsies).

Opaque meal shows that the mucosal marking in the duodenum and Jejunum are coarser and disturbed; the bowel is full of gas; the meal is distributed in segments of the gut isolated in distended coils on account of disturbance of motor activity which is the result of deficiency states. The passage of meal through the gut is irregular and delayed; the peristalsis is inhibited.

(4) Clinical aspects

The onset of the disease is usually insiduous. In most of the cases three cardinal symptoms—diarrhœa, flatulent indigestion and sore tongue and mouth are present when the disease is fully established. These may appear simultaneously or one after the other in any order. Soreness of mouth often preceeds diarrhoea. Skin changes may occur and are of two types—follicular hyperkeratosis and parakeratosis. Well marked skin pigmentation especially of the face occurs in some cases.

In the early stages diarrhoea may be intermittent and mild. The diarrhœa is of two types, acute and chronic; in the former the stools are watery. Generally after food which disagrees, the abdomen becomes distended with gas, there is gurgling and a liquid stool with much gas is passed giving much relief. There is no pain or tenesmus. Three or four such stools may occur in the course of the day. This has been termed as 'pre-sprue' stage by some. The acute stage passes off and is followed by chronic sprue stools, which become pasty, light yellow or clay coloured or white frothy and bulky in appearance and with a foul .smell. There may be only one stool early in the morning, but often there are several in the day. Microscopically the appearance of the stool is normal except that there is much fat and crystals of fatty acids. In sprue the reduction of the normal stool pigment is a classical feature and all cases at some stage or other have passed pale stools.

Flatulence always occurs. It is at first mild and intermittent and may be relieved by passage of stools. It gradually gets increasingly severe and may cause destressing, painful and persistent abdominal distention which is most troublesome in the latter part of the day and attaining its maximum in the evening. The soreness of the mouth increases, the tongue and buccal mucosa may develop small apthous ulcers; later the tongue becomes red, acutely inflammed and denuded. Salivation may be troublesome. The buccal lesions may extend downwards into the pharynx and oesophagus and swallowing may be very painful and even dysphagia may occur.

The disease usually progresses by spontaneous remissions, followed by increasingly severe relapses. The patient gets extremely emaciated and mentally depressed. Anæmia sets in after diarrhoea has lasted for sometime. Anæmia is at first of the hypochromic microcytic type, but later of hyperchromic macrocytic type indistinguishable from pernicious anæmia rapidly develops. The red cell may fall from two to one million per cmm. This is manifested by increasing weakness prostration, disinclination for exertion and other symptoms of anæmia. The patient becomes progressively emaciated from lack of absorption from the gut and consequent starvation. The blood pressure is low (90 to 100 mm) and basal metabolic rate of minus 10 to 20. Nervous symptoms referable to subacute combined degeneration of the spinal cord may become manifest. The blood calcium is low and if severe, tetany may develop. Symptoms of various vitamin deficiency states may become evident.

(5) Diagnosis

The diagnosis presents little difficulty once it is realised that dysentery is not the only cause of diarrhœa in the tropics. The characteristic combination of steatorrhea, glossitis and a macrocytic anæmia makes the diagnosis easy. In the stools, there is a large excess of fat. The blood calcium may be low, and the glucose tolerance test gives a flat blood-sugar curve due to delayed and defective absorption.

Gastric analysis often shows hypochlorhydria or achlorhydria with response to histamin. More than half of the cases have acid curves at least within normal limits. Presence of hydrochloric acid in stomach distinguishes it from pernicious anæmia. Also there is no jaundice in sprue while in pernicious anæmia the skin is lemon yellow in colour.

Hypoprotinaemia is common especially in advanced cases. Sprue is distinguished from pellagra by the fact that there are no cutaneous manifestations and steatorrhea.

(6) Treatment

In the treatment of sprue utmost care lies in procuring bodily rest and selecting a very careful dietary for the patient. It is to be understood that sprue is a disease in which the gastro-intestinal tract bears the greatest brunt of attack. Hence if the treatment of the case is begun early in the disease by careful regulation of the diet, very successful results are obtained, while if it is undertaken too late in the period when the absorbing surface of the intestine is damaged the results are discouraging. The treatment must be thorough and persistent. It should be realised that carefully adjusted diet does little more than rid the bowel of a mass of material it is unable to absorb. The deficiency syndrome can only be corrected by administering vitamin preparations and liver extract. Food should, however, be given by the mouth in form of diet rich in proteins and adequate in calories. This requires considerable efforts on the part of nursing staff since patients have lost all appetite and desire for food. In fact the treatment depends entirely on intensive liver therapy. While refined liver extracts are effective in pernicious anæmia they have relatively little value in sprue. Crude extract may be given by the mouth in mild cases, but injections are preferred. Very much larger doses of crude extracts have to be given than are given in pernicious anæmia. It is advisable to give 5 ccm every other day or every day in the first week or two of treatment till the patient shows marked signs of improvement. The dose is then reduced to 5 ccm twice a week till he completely recovers. After the acute symptoms are controlled liver extract in doses of 5 ccm weekly may have to be continued for months or years before it can be stopped altogether. In very serious cases with marked degree of anæmia a blood transfusion of 500 ccm of whole blood should be given and if necessary repeated.

The diet during treatment should be low in carbohydrates and fats and high in proteins. Meats are well bourne and should be given in the beginning and later carbohydrates are introduced in form of potatoes and other starchy vegetables. Later fruit and vegetables are given in small quantities and

gradually the patient is got on to normal dietary. Vitamins particularly A and D are given as also vitamin B complex in form of marmite. The results of liver treatment are remarkable.

Recent work has shown that niacin (nicotinic acid) checks diarrhœa and relieves the condition of the mouth and it also has similar effects in pellagra. Niacin occurs in crude liver extract in large quantities and in some specimens as much as 500 mgm per cent is present, in highly refined liver extracts its percentage is much reduced. The treatment of sprue with niacin is still in experimental stage.

(7) Prognosis

Complete recovery can be made in about six months in ordinary cases if treatment is properly carried out. The cases which give rise to anxiety are those which are profoundly emaciated, in which asthenia is associated with low blood pressure, the systolic pressure falling below 80 mm. They show, to some extent, signs of dehydration, but hydration does not produce much improvement. Plasma transfusion appears to have been advantageous to saline transfusion.

Folic acid is the name given to a pure chemical substance isolated originally from spinach; it is found in many other green leaves, including grasses and in mushrooms, liver and yeast. It takes its place among vitamins and is fully discussed in that section. Folic acid has been synthesized and the synthetic product has been used successfully in the treatment of pernicious anæmia, nutritional macrocytic anæmia, sprue and macrocytic anæmia accompanying pellagra. It can be given by the mouth or parenterally dissolved in disodium phosphate. In sprue 20 mgm are given daily by the parenteral route with good results. As much as 400 mgm have been given by the mouth without untoward effects. Folic acid therapy is still in experimental stage.

9. Lathyrism

Lathyrism is a disease caused by the ingestion of the seeds of a vetch of the genus *Lathyrus* and is characterised by spastic paraplegia resulting from degenerative changes in the spinal cord. The disease is common in certain parts of India, especially the United Provinces, the Central Provinces and several districts of Behar.

In 1868 Irving stated that nearly 7 per cent of the population of certain districts of the Central provinces were afflicted with the disease and Acton in 1922 found that in Rewah there were 60,000 cases chiefly affecting men in the early prime of life.

(1) Aetiology

An exogenous agent appears to be the cause of this disease. In India it has been held for a long time that lathyrism is due to certain poisonous substances occurring in the pea *Lathyrus sativus* (*Khesari dal*). Buchanan in 1904 stated that continued consumption of *khesari dal* for some months together contributed to the causation of the disease. Lathyrus is a vetch, and the peas of three species, *L. sativus, L. clymenum, L. cicera* are used habitually as an article of diet in India and Algeria by the people. When consumed in moderate amount they do not produce any symptoms, but in times of famine increased consumption has given rise to epidemics of severe poisoning especially among the poorer classes who subsist on them. Besides India, outbreaks have also occurred in Italy, France and elsewhere in Europe. Occasionally similar symptoms have been produced in cattle. In the laboratory, feeding experiments give varying results as different species of animals vary greatly in their susceptibility to the poison. Fowls, pigeons, and partridges eat the lathyrus pea freely and with apparent immunity; ducks are readily affected. Some animals fatten on this pulse while others are immune. Horses are particularly susceptible

and have often died. The outstanding symptom in animals is muscular weakness; in acute poisoning with large doses the motor nerves are depressed but in feeding experiments with repeated doses such an effect may not be observed. In chronic poisoning the deep reflexes are increased and in monkeys and horses spasmodic attacks may occur irregularly in addition to the paretic symptoms. Death results from paralysis of the respiratory muscles.

Though the disease has been attributed for a long time to the consumption of the lathyrus pea in sufficient quantity over a sufficient period of time, the exact factor responsible for its production is not yet settled. Acton (1922) describing some cases in Rewah stated that *Lathyrus sativus* peas caused paralysis and death in ducks, but after the pulse had been steeped in water the animals lived and thrived on it. This is due to the fact that the poisonous principle is soluble in water, and can be removed by soaking the grain for 24 hours in three changes of water. It has been found, however, that some crops will apparently produce lathyrism while others will not and the experimental work by various workers has given discordant results as to the poisonous properties of lathyrus. Howard, Simonson and Anderson (1925) in an elaborate study of the subject concluded that *khesari dal* (*Lathyrus sativus*) did not contain any poisonous bases at all, and did not produce symptoms of paralysis in ducks that had been fed exclusively on this vetch, provided it had been freed from a closely allied vetch, *Vicia sativa* (*akri or akti*). When *Vicia sativa* was given to ducks and monkeys in the proportion of 10 to 50 per cent in the food, it produced symptoms of nervous disease very similar to those of lathyrism, followed by death. They concluded that seeds of *Lathyrus sativus* are chemically free from any poisonous alkaloids and are harmless and even nourishing to these animals. Though some of the symptoms in ducks resemble those occurring in human lathyrism they are not prepared to state, in the absence of definite pathological proof, that *akti* is the cause of *lathyrism* in man.

Acton and Chopra (1927) reinvestigated the problem of the part played by *Lathyrus sativus* in the causation of lathyrism; they concluded that lathyrism is due to a contaminating weed *Vicia sativa* in *khesari dal* and not to any poison in the latter. The non-germinating seeds of *Lathyrus sativus* freed of *akti* likewise produce no toxic symptom in experimental animals. The toxic principle divicine resides in the contaminating weed. This is probably closely related to barbituric acid which forms a toxic principle in many drugs such as, veronal, luminal, etc. Divicine can be split into barbituric acid by hydrolysis when the two NH_2 groups are replaced by an H and O group and it is probably due to this hydrolysis of barbituric acid that some toxic substance is formed which acts on the central nervous system. It has been found that doses of 50 mgm of this toxic base can produce paralysis of the hind legs of guinea-pigs. The reason why lathyrism is not prevalent throughout the year is the fact that in ordinary years *khesari dal* is free from this contamination and hence no lathyrism occurs, but during the period of famine the diet of poorer classes of people is wholly made up of an imported variety of lathyrus contaminated with 'akti', as it is the cheapest food available at the time. Moreover when a deficient diet, such as a whole diet of khesari dal is consumed, the general resistance of the body is lowered so that the poison can act more powerfully on the individuals.

Some observers are of opinion that some other factor besides the presence of lathyrus seed in the diet, is responsible, and that lathrism may be to some extent a deficiency disease. Degenerative changes occur in the spinal cords of dogs fed on diet deficient in vitamin A, and it is possible that a deficiency of vitamin A allows the neurotoxin in the peas to exert its harmful effects on the central nervous system. In India the author has not come across any case which has not given the history of using *Lathyrus sativus* seeds as a staple article of diet.

(2) Pathology

There is a well marked degeneration of the direct and crossed pyramidal tracts, and in the column of Goll. Sclerosis of the lateral region of the cord has been observed, not strictly confined to the pyramidal tract, throughout the lower cervical, dorsal and upper lumbar cord. The small blood vessels may be sclerosed and obliterated in places. In horses, well marked degenerative changes have been described in the cells of the grey matter of the cord, in the vagal and accessory nuclei of the medulla, in the lateral columns and motor nerve roots of the spinal cord and in the recurrent laryngeal nerves, with thickening of the walls of arterioles and capilliaries of the spinal cord.

(3) Symptomatology

The symptoms closely resemble those of spinal sclerosis. In man the symptoms are usually precipitated by exposure to cold, wet and fatigue. Sometimes there may be prodromal symptoms, such as pain, numbness and cramps. As a rule the patient suddenly feels the

legs and loins heavy; there is dragging of the legs, and more or less inability to walk. Later, there may be extreme spasticity and rigidity of the leg muscles; the case may progress to such a condition that he can only move his hands and feet in a sitting posture. Sensory disturbances are not generally evident. The sexual power is said to be enfeebled, but the mind, speech and pupils escape.

The physical signs are those of typical spastic paraplegia, with exaggerated knee and ankle jerks, ankle and patellar clonus and an extenser plantar response. The gait depends on the degree of involvement of the spinal tracts. At first the attitude of the legs is one of extension with adduction, the patient walking on tiptoe; the body is raised high before the toes leave the ground, giving rise to up and down movement of the shoulders, and progression is affected by tilting the pelvis and circumducting the legs. The legs may be crossed scissors-wise. The more feeble patients use one or two long sticks for support in walking. Later paraplegia develops, and in the final stage walking is impossible. Progression is then made by crawling on the balls of the toes and on the hands, which are often supported by wooden sandals. In most cases, the trunk and upper limbs are unaffected.

The disease runs a very chronic course. Degeneration being limited to the motor tract is not a direct danger to life. It may be arrested but there is not any possibility of cure in the sense of restoration to the previous condition of health. The degree of paralysis produced by the original attack remains permanent.

(4) Diagnosis

Diagnosis is made clinically together with the dietetic history. The disease must be differentiated from other causes of spastic paraplegia such as syphilitic myelitis, spinal caries, subacute combined degeneration of the cord, amyotrophic lateral sclerosis and syringomyelia.

(5) Treatment

There is no cure for this disease as the changes produced in the cord are permanent. Jacoby (I.M.G, 1945, Page 246) has, however, obtained successful results in a small series of cases with prostigmin which is the synthetic equivalent of the alkaloid physostigmin. He injected 0.5 mgm of prostigmin methyl sulphate in 1 ccm of water intramuscularly every day in series of 14 patients. Of these 8 patients who received 12 injections each improved considerably. All pains disappeared. The muscular rigidity and spasm in the lower limbs were relieved. Three cases improved and could walk but had ataxia on closing their eyes; the pains disappeared. Three cases did not improve at all. Jacoby considers that in lathyrism the anatomical functional lesion is in the upper motor neurons of the pyramidal tract; there is imbalance between the production and destruction of acetylcholine, thus interfering with the transmission of co-ordinated nervous impulses in the tract and producing the symptoms of lathyrism. Prostigmin acts by protecting acetylcholin against destruction and the impulses thus become co-ordinated.

The prevention of the disease depends on the improvement of the economic condition of these people and abolition of the system of bondage that exists in some states of India at present. Howard suggests that *khesari dal* should be planted in drills and the contaminating *Vicia sativus* removed by weeding. This should be done not only in places where lathyrism is rife but also in those areas from where the imported grain is sent to the famine stricken areas. But in view of the divergent views on the part played by vicia in the spread of the disease, no successful measures can yet be devised. Nourishing diet is obviously of value. There is no specific treatment. The paralysed parts should be kept warm; massage and electricity are indicated.

CHAPTER XI

CLIMATE, WEATHER AND MAN

THE INDIVIDUAL AND HIS ENVIRONMENT: PHYSIOLOGICAL EQUILIBRIUM, MECHANISM OF ADAPTATION, ATMOSPHERIC ENVIRONMENT—CLIMATE AND THE INDIVIDUAL: EFFECT OF HEAT AND SUNLIGHT IN THE TROPICS, EFFECTS ON THE DIGESTIVE SYSTEM, EFFECTS ON THE RESPIRATORY AND CARDIOVASCULAR SYSTEMS, EFFECTS ON THE NERVOUS SYSTEM, EFFECTS ON THE ENDOCRINE SYSTEM AND GROWTH, BASAL METABOLISM IN THE TROPICS—TROPICAL CLIMATE AND RESISTANCE TO INFECTION—CLIMATE HEALTH AND DISEASE: TELLURAL INFLUENCE—TROPICAL NEURASTHENIA: AETIOLOGY AND INCIDENCE, TREATMENT.

Reaction of Body to Climate

Introduction.

Though physiology in its widest sense signifies the study of the phenomena presented by all living organisms, its chief interest is centred round the body of man. It should therefore be the objective of human physiology to study the so-called 'normal' human individual, since it provides an essential base line for the study of disease. The definition of 'normal' and the criteria of what constitutes the normal are still an open question. As the human individual cannot exist uninfluenced by his external environment and as the environment is variable the question of normality of the individual becomes relative, and has to be studied under the varying conditions of the altered and continually altering environments to which the individual must necessarily be subjected in different parts of the globe.

1. The Individual and his Environment

Historical.

The study of the individual in relation to his environment has recently engaged the attention of many a physiologist and clinician though the problem is by no means a new one. In the earliest days of medicine in India centuries before the Christian era, the Hindu physician *Charaka* drew attention to the effects of different 'airs' and places on mankind. He prescribed special diet for particular seasons of the year, recommending one type of food for the summer and another for the winter. In the early dawn of Greek medicine Hippocrates recognized not only the significance of a 'change of winds' but also its physiological implications. The interest in this subject waned in the 19th century when the science of bacteriology made its beginning through the pioneer researches of Pasteur, Lister and others. In the study of diseases, emphasis was laid more on the invading parasite than on the invaded individual. With the advent of the science of immunology, serotherapy and chemotherapy, the importance of environmental influence receded more and more into the background. Though the interest of medicine at present is chiefly centred, and perhaps rightly, on infectious diseases, the study of environmental conditions is none the less important. Let us consider how the individual reacts to his surroundings. Two methods have been devised by the organism to meet the forces of the environment. It seeks to lessen the impact and to meet the shock by increasing the resistance, in an endeavour to prevent disturbances of its equilibrium. This is usually effected by diminishing the consumption of energy or, in other words, the organism becomes *less reactive*. This phenomenon of attempting to shield itself against invasion is exemplified in spore formation and in the various forms of encystment of lower organisms. In the higher organisms, the same reaction finds expression in the mechanism of hibernation, in the deposition of subcutaneous fat and in the development of the epidermis. The other alternative is that the organism becomes more *reactive* and, instead of resisting any force disturbing its equilibrium, seeks to attune its mechanisms of equilibration to such a degree that they swing in a rhythm with the environmental force. This is seen in the development of special mechanisms for the rapid mobilization of reserves, in the unusual acuity of receptors and not infrequently in the formation of special sense organs. The history of man is not inaptly described as 'an endeavour first to adjust himself to his environment and later to control that environment itself.'

Reactions to environment.

(1) Physiological Equilibrium

External and Internal Environment.

The external environment with the effects of which we are concerned is not in reality the one in which the body cells live. As is well known, though the external environment varies greatly, the internal one remains remarkably constant and this is achieved, firstly, by certain physical and chemical factors inherent in the saline fluids which constitute the internal environment—the *milieu interne* of Claude Bernard, and secondly, by certain bodily reactions designed to prevent abnormality. The extent to which the organism will respond, when brought into play by adverse changes in the

environment of the individual, and the degree of their response are measures of the organism's reserve capacity or power of adaptation. On this reserve power depends the success of the individual in his adaptation or acclimatization to changed surroundings and environments.

(2) Mechanism of Adaptation

Physiological factors in adaptation.

Adaptation has been aptly defined as the continuous adjustment of internal relations to external relations. The ability to respond actively to changes in environment is an essential characteristic of any organism. The range of environmental adaptation of even the most primitive cell is relatively wide, although the mechanism of response may, be simple; but as we advance in the scale of animal life, the range of adaptation becomes even greater as the mechanism and the means of adjustment become more complex The higher we ascend in the animal scale, the means by which the organism responds to environmental changes become more varied and complex and an additional mechanism of adjustment other than chemical and hormonic is called for. For this purpose, the anatomically-defined autonomic *nervous* system, both sympathetic and parasympathetic, has been developed. Finally, when the level is reached at which reason is developed, not only are accomplished changes appreciated but the possibility of alteration, made or threatened, can be recognized and guarded against; in other words, the adaptive reactions become conscious and adjustment to meet them 'volitional'. All the reactions of a biologic mechanism to environment may be said to be mediated through three distinct but mutually-related and integrated processes—chemical, endocrinal and nervous. The three components are functionally inseparable, and no one component can be disturbed without disturbing the others.

Reserve force of Organism.

The ability to maintain an equilibrium without the breakdown of the adaptive mechanism under adverse conditions determines the development of the individual and ultimately of the species. The greater the possibility of protective reaction the greater are the chances of survival. It seems important therefore to stress the fact that the animal body, although often compared with a man-made engine, differs fundamentally from the latter in that it has latent reserve forces capable of adjusting the animal to a new environment, in other words it possesses powers of adaptability. These forces call forth hypertrophy of glands and tissues in response to appropriate stimuli and even carry out repair of damaged parts. Sensibility or facility of response can be improved by frequent usage or 'training', as in the case of muscular exercise. The athlete can undertake a given amount of exercise with less disturbance of respiration and circulation than can the untrained person, and his return to the resting normal condition is also quicker.

(3) Atmospheric Environment

The most important environmental factor is the atmosphere in which we live. As the atmospheric environment is so familiar, we fail to recognize the importance of the part it plays in moulding the physiology and sometimes the pathology of 'air-living' animals. We are likely to forget that we take in as much air by weight per day as we do of food. The atmosphere or the air-mass surrounding the earth is not stable but fluctuates from place to place and from time to time, particularly with the 'cyclonic' waves. These fluctuations and atmospheric disturbances are normal and necessary sequences to temperature differences on the earth's surface.

Climatic factors.

The term 'climate' has been defined as the combined effects of the sun, the atmosphere and the earth upon living objects at any one place on the earth's surface. Climate primarily results from the effects on the earth's surface of the incident solar energy and its unequal distribution. The climatic elements temperature, humidity, etc. are dependent on physiographic factors, such as distribution of land and water, mountain ranges, nature of soil, distance from the equator and height above the sea level. The latitude and the altitude are, however, the most important and deserve special attention.

The words 'climate' and 'weather' are often used synonymously in common parlance but 'weather' means those atmospheric elements we see, feel or observe at a particular time or specified period, while climate means the aggregate of weather conditions. In cold and temperate latitudes, the weather becomes a more outstanding feature than the climate itself. In the tropics, for the greater part of the year, the climate becomes a succession of equal combinations of meteorological elements, the annual range of which seldom exceeds the diurnal range. The 'weather' in the tropics therefore is practically the same as the 'climate'. The monotomy of the tropical climate is a characteristic not seen in the temperate or cold regions of the globe.

Tropical climates.

It is generally assumed that the climates of the whole of the tropical zone are in all respects alike and therefore the term 'tropical climate' is often used, though incorrectly. Climatologists have divided the tropical belt into several subdivisions according to the situation of the land in relation to the equator, the vicinity of water, the growth of vegetation, the presence of mountains or hills, the proximity of land to oceanic currents and trade winds, and its height above the sea level. All these factors will naturally influence the 'climatic type' of a locality. Thus the tropical zone includes deserts, damp forests, jungles, swamp lands and fertile islands refreshed by cool steady winds from the ocean, low-lying plains and valleys under the influence of the periodic monsoons, and mountains several thousand feet above sea level. All these regions are not equally disagreeable for the residents. The deserts, for example, are free from many tropical diseases, for their parched soil and dry, sterile air are unfavourable to the development and distribution of most micro-organisms and disease-bearing insects. On the other hand, the dust, heat and brilliant sunshine are very trying, and the paucity of water is obviously a serious limitation. Again, wherever mountains and plateaux occur in the tropics, the climatic conditions are distinctly different from that in the plains and may be actually mild and agreeable. It will therefore be seen that, within the tropical zone, there may be areas with peculiar climatic types with their special advantages and disadvantages.

The climate in tropical plains varies but little and the temperature ranges between 75°F (25°C.) to about 115°F. (46°C.). There are no seasons in the tropical zone and monthly and diurnal variations of temperature are slight. These areas are often subject to the influence of monsoon with their alternate dry and wet spells. They are usually very fertile and support a variety of luxuriant vegetation. A large portion of the plains of India has a climate of this type and therefore it is of very great interest to us to know the physiological adjustments required of human beings in such a climate.

2. Climate and the Individual

Climate and Civilization.

The influence of climate on man is so well recognized that it is often the main topic of daily conversation. One often hears of a 'bracing' climate or a 'relaxing' climate, and the beneficial effect of climate variation finds its expression in our desire for a 'change of air'. There appears to be a direct relationship between the physical and mental vitality and the variability of climates. Huntington in his book on 'Civilization and Climate' puts forward the theory that all the world's great civilizations are now in regions experiencing often unexpected changes of weather and that the superiority of races inhabiting temperate regions of the globe is largely the result of climatic influences. Mills (1934 to 1936) has corroborated some of Huntington's conclusions. According to him, there are energy differences between the races living in warm climates and those in temperate climes which cannot be accounted for on the basis of diet and mode of living alone. Some psychologists maintain that each climate tends to develop its own characteristic mentality. In other words, climate not only modifies the physical features but also the nervous system of the individual. Though man can live in any region where food and water can be obtained, he can best develop his physical and mental capabilities under the most favourable environmental conditions. There seems but little doubt that there is also a distinct optimum condition for man, just as for plants and animals, and any departure from it adversely affects the organism.

(1) Effect of heat and sunlight in the Tropics

In considering the effects of residence in tropical climates, attention is at once directed to three major environmental factors, *viz, temperature, humidity* and *sunlight.* There are a number of other climatic and environmental factors, such as wind movement, atmospheric electricity, ionization, etc., which also affect the human organism, though the responses elicited do not leave lasting impressions on the individual. In nature, all these climatic factors operate simultaneously and the reactionary changes observed at any one time are in most cases a summation effect, one factor being superimposed on the other. Thus while the acute effects of encountering very high temperatures as of a heat wave in a desert region may be tolerated without harm, the organism may show signs of intolerance or breakdown when excessive humidity is superimposed on such a high environmental temperature.

Sunlight.

The biological effect of sunlight has received perhaps more attention than any other factor in this group. A careful perusal of the literature shows that the knowledge gained in this branch is indefinite and unsatisfactory. The beneficial results obtained by heliotherapy in tuberculosis and the effects of ultraviolet light in the treatment of rickets created the impression that the ultra-violet portion of the spectrum was the important factor responsible for the biological effects produced. Several investigators in the tropical belts of Australia, the Philippines and the Netherlands East Indies have adduced

experimental evidence indicating that the ultra-violet light, being nonabsorbable by the skin, is probably much less important than the infra-red and the visible-red end of the spectrum, which penetrates the superficial layers of the skin and is perceived by the organism as heat energy. Whatever direct effects tropical sunlight may have, have therefore been attributed to its thermal action.

Humidity.

The effect of humidity on the human organism has recently engaged the attention of public health authorities and sanitary engineers interested in environmental control through air-conditioning methods. That humidity modifies, to a great extent, the effects of heat is generally recognized. A moist humid atmosphere is associated with depression and discomfort, and, conversely, dry air, irrespective of its temperature, induces a feeling of comfort and well-being. The water content of the atmosphere must affect the water content of the body. In the regions of high environmental temperature, such as are met with in the plains of India for example, greater humidity will prevent proper evaporation and heat loss with resulting disturbance in the respiratory exchange, in the carbon dioxide content of the blood, and consequently in the water and ion balance. It may, therefore, be stated that high humidity, by diminishing evaporation of sweat, merely aids in increasing the effects of heat, but as a direct factor it does not appear to possess any great specific importance.

Temperature.

Studies on the changes produced in the body of man as a result of exposure to high environmental temperature have been numerous, but, like most aspects of tropical physiology, the data are conflicting. It has been reported that a change in the environmental temperature produces a change in the *body temperature* of the individual and that in the tropics body temperature may be permanently above the average in the temperate zones. While it is true that a change in the body temperature of an individual may be easily effected for brief periods by exposure to high external temperatures, it is doubtful if, in view of the sensitive heat-regulating mechanism in man, a permanent change can be brought about by prolonged exposure such as residence in the tropics. From physiological considerations, it is logical to assume that the flushing of the skin, as a result of exposure to a hot environment, raises the skin temperature, but the temperature soon drops when perspiration breaks out.

Role of skin pigment.

It was generally recognized that tropical races are at an advantage over the European settlers in the matter of heat dissipation. The possibility suggested itself that acclimatization for generations in a climate where the mechanism of heat loss had to operate under excessive strain might have endowed the skin of the tropical races with an additional power and facility in getting rid of excess heat by the processes of physical heat regulation—conduction, convection, radiation and evaporation—at a more rapid rate than individuals from cooler zones. It has been claimed that the skin of tropical races loses heat by convection more rapidly than the skin of white races but these conclusions have not been universally accepted. The suggestion next put forward was that the dark skin radiated heat more quickly than the white skin, but satisfactory evidence has not been obtained in favour of this contention. There is little doubt that pigment plays an important part in heat regulation in other ways. Pigmentation of the skin is necessary to protect the deeper structures from the injurious effect of sunlight and to protect the sensitive cutaneous nerve endings from irritation. The brown-skinned races in the tropics, by virtue of the pigment, have apparently developed a more sensitive cutaneous heat-regulating mechanism, and they can cool off more rapidly by reason of the early formation of a fine layer of sweat drops.

Sweat secretion.

For physical heat regulation, however, the activity of the sweat glands is of outstanding importance. Their efficiency depends on several factors: (1) humidity of the environmental air, (2) concentration of sweat glands per unit area, and (3) the degree of activity of these glands. Humidity, though it is important, because on it depends the evaporation of water from the skin, does not in actual practice exert a great influence, because apparently high degrees of humidity, provided they fall short of absolute saturation, can be borne by the system with impunity. The concentration of sweat glands in the skin may regulate the amount of perspiration, and it was believed by many early workers that the better heat-tolerance of the dark-skinned races was probably due to this factor.

Composition of sweat.

A study of the composition of sweat produced in extreme dry heat shows that there is an adaptive response on the part of the organism and that the concentration of salt in sweat decreases after the first few days in the hot environment. To cite a concrete example, the amount of sweat produced with ordinary type of work in a climate such as that of Calcutta (95° to 105°F. or 35° to 41°C) may be as much as 7 litres during the day. Assuming a sodium chloride concentration of 0.3 per cent, this means a loss of 22.5 gm of salt. Man's daily intake of sodium chloride is 10 to 15 gm and since the average normal excretion of salt in the urine is in the neighbourhood of 12 gm in 24 hours,

the degree to which muscular work in such a climate may deplete body chlorides is remarkable. If this depletion is not compensated, the normal acid-base equilibrium of the body fluids is apt to be seriously disturbed and heat cramps from loss of salt may supervene. It seems clear that some sort of adaptation takes place in normal healthy individuals. Little is known, however, of the nature of this adaptation, though it has been noted that all men sweat more readily as they get accustomed to exposure to heat. This increased 'ability to perspire' is known to be one of the few definite adaptations to high temperature and it is interesting to note that it is a case of 'controlled' rather than profuse wasteful sweating. The sweat secreted is said to contain an unusually low concentration of sodium chloride and this would appear to be an attempt at conservation of chlorides, as even on a high chloride intake their concentration remains low.

(2) Effects on the Digestive System

Atonia and stasis.

It has frequently been observed by Europeans coming to the tropics that there is a definite impairment of appetite and a lessened desire for animal food. Though this may indicate a diminished power of digestion, it is probable that the disinclination for animal food may be an adaptive response to a lower caloric need in the tropics. There is also a tendency to an atonic condition of the gastro-intestinal tract, as evidenced by an increased tendency to stasis with resultant constipation and gaseous distension. This may be due to sedentary habits and lack of exercise though it is perhaps largely due to the fact that Europeans in the tropics are apt to follow their old dietary habits suited to temperate climates and do not take enough carbohydrates, vegetables and water to provide for the roughage. On the other hand, it is possible that the high environmental temperature in the tropics leads to a vaso-dilatation of the skin capillaries and a reflex vaso-constriction and anæmia of the splanchnic area, resulting in diminished gastric and biliary secretions, and possibly also in an atonic condition of the stomach and intestines. With skin stimulation, the activity of the mucous membrane of the stomach and gastro-intestinal tract is decreased, but with lessened activity of the skin (as with cold) the functional activity of the mucous membrane of the gastro-intestinal tract is increased. It is probable that the inhabitants of warm climates make an appropriate physiological and dietetic accommodation by the greater use of condiments. Spices in solution are known to increase the activity of the villi and secretory glands, and the free use of condiments in the tropics may be an expression of the greater need for gastro-intestinal stimulation where skin activity is normaly unduly accentuated.

Gastric acidity.

A general impression seems to prevail that the gastric acidity of rice-eating Indians is lower than that of meat-eating Europeans. A fractional gastric analysis on a number of normal Indian male and female subjects showed that on the whole their gastric acidity was higher than in normal Europeans. This is contrary to expectations, for hot climate and vegetarian diet are both believed to be factors that tend towards decreasing gastric acidity. No explanation of this phenomenon is yet forthcoming.

Bacterial flora.

Bacteriologists have claimed that the bacterial flora in the intestines of inhabitants in the tropics are different from those of colder climes. The bacterial flora of the lower intestinal tract vary with external temperatures and hence it is natural to expect a difference between those found in the tropics and the cooler regions. As the nature of the food is also known to influence the relative proportions of the various bacterial groups in the intestinal flora, and as the predominant dietary constituent in the tropical inhabitants is carbohydrate, the claim has much in its favour. Workers in this field appear to agree that an animal protein diet encourages the growth of proteolytic types, whereas the addition of lactose and dextrin stimulates the development of aciduric micro-organisms. Normally, a sufficient amount of lactose in most instances will cause a complete transformation of an ordinary mixed flora into one consisting predominantly of streptococcal and lactobacillary types. It has, for instance, been recently shown that banana powder, apple powder,and raisins, when fed to white rats subsisting on a high protein diet,consisting exclusively of raw chopped beef, produce a change in the intestinal flora from one in which nonaciduric bacteria predominate to one in which *Lactobacillus acidophilus* is the outstanding organism. It has been further pointed out that the simplification of the intestinal flora varies directly with the hydrogen-ion concentration, a pH of 7.0 being characteristic of a gas-producing proteolytic type, whereas an increasing acidity is characterized by a diminution of proteolytic types and the replacement by the aciduric types. It is evident, therefore, that a diet containing sufficient carbohydrate is capable of effecting a complete turnover from a heterogenous Gram-negative type of flora in persons fed on high protein diet, to a flora markedly simplified and Gram-positive, inducing coincidently a change in the hydrogen-ion concentration from nearly neutral to distinctly acid.

pH of faeces.

Recent work on the hydrogen-in concentraton of fæces of different classes of persons living in Calcutta shows that, whereas the reaction of the stools of most of the Bengalis is acid, those of the Europeans, Anglo-Indians and Marwaris are mostly alkaline. Although the Marwaris are vegetarians and live on a predominantly carbohydrate diet, they consume very little rice and take more wheaten bread (*chapati*). It would appear that the consumption of rice produces an acid reaction in the large intestine while wheat produces an alkaline reaction. When Bengalis were put on bread, and rice was cut off, the reaction of the stools changed from acid to alkaline in most case. In view of the acidity of the stools, it was thought likely that aciduric organisms would predominate in the stools of Bengalis, but surprisingly enough *Lactobacillus acidophilus* could not be isolated.

(3) Effects on the Respiratory and Cardiovascular Systems

Respiration.

There is a good deal of divergence of opinion with regard to the effects of tropical climate on respiration. The consensus of opinion appears to be that the rate is diminished but the depth, as indicated by measurements of the minute volume, is increased. A slightly deeper breathing is more efficient for the individual economizes energy and, at the same time, the increase in the volume of air breathed cools the system. These changes in the rate and vital capacity are probably of a temporary nature, evident only in the case of the white immigrants to the tropics, and are not apparent in the natives of the tropics.

Blood pressure.

A slight decrease in the pulse rate and in systolic blood pressure is a very common finding in the tropics. Mills (1936) has shown that people migrating from West Central Europe or Central North America to the tropics nearly always suffer a marked fall in blood pressure within a year or two, even though no debilitating disease or infection has occurred. Probably there is a slight decrease in blood pressure due to lowered vasomotor tone and a general slowing of physiologic activity in the high environmental temperature in the tropics, but the changes are comparatively insignificant. The increase in sweating which is necessary in the tropics must require a constantly increased blood supply to the skin, and has been estimated to be as much as 50 times of the amount in temperate climes. The immediate result, if the total volume remains constant, would mean relative anæmia of the splanchnic region and the vital organs in the abdomen. To counteract such circulatory disturbances there is usually an increase in the total blood volume.

Haematological standards.

The pale complexion of the white races migrating to the tropics has been frequently referred to as an indication of anæmia or 'thinness of blood'. Most authorities are of opinion, however, that a tropical climate *per se* does not produce true anæmia. Several workers in the Dutch East Indies found that the values for red blood cells and the hæmoglobin lay more or less within the same limits for the white sojourner and the native resident of the tropics. In two series of normal healthy Indians consisting of 50 and 30 individuals the mean hæmoglobin contents were found to be 14.77 gm and 15.70 gm respectively. With regard to the number of red cells, the average figures obtained in the Calcutta series were 5,533,000 and 5,362,000 and the mean of the Bombay series was 5,110,000. These figures are again above the classical 5,000,000.

Most hæmatologists in the tropics appear to agree that the white blood cells are generally decreased though the change is not very great. As a rule the polynuclears have been found reduced and the Arneth count shifted to the left. This has been fully confirmed by workers in Iraq and in Calcutta. It was found that the number of leucocytes, if it differed at all, was slightly lower than in temperate climates, but the eosinophile percentage was definitely higher. In 50 normal city-dwelling Indians, who had no heavy helminth infections, the mean eosinophile percentage was nearly 7.0. Not only is the normal eosinophile count of the Indian high, but in disease the eosinophilia tends to attain a very much higher proportion than in the Europeans in temperate climates, *e.g.*, 60 to 80 per cent eosinophilia is not uncommon in asthma and other conditions.

It is not possible, in the present state of our knowledge, to explain the significance of the different hæmatological responses observed in the tropical races. From the findings of various workers it seems reasonable to conclude that, far from interfering with the functions of the erythropoietic organs, the tropical climate may actually exert some stimulating action on these organs. The possible bearing of the low average white cell count on the defensive mechanism against disease processes requires further investigation.

Biochemical changes in blood.

There is little doubt that there is a more pronounced disturbance of water metabolism and water movement in the tropics than in temperate surroundings. This will naturally be reflected in the water content of the blood. A number of observers have

attempted to throw light on this important aspect of tropical adaptation by measurement of blood chloride concentration. Sundstroem (1925) working in Australia found that the blood plasma was more concentrated than normal and richer in chloride content in a hot environment. A slight alkalosis has been reported particularly in persons of sedentary habits which can be removed by water intake and muscular exercise.

The average concentration of serum proteins in the blood of Indians according to Chopra and Mukerji (1931) appears to be slightly lower than the accepted standard figures of Europeans, but the albumin-globulin ratio remains unchanged. The results with regard to non-protein nitrogen content are not conclusive. Since protein intake is likely to be low in the tropics, this will probably be slightly lower than normal. The lipoid constituents of the blood including cholesterol have generally shown lower values. Blood sugar apparently is not so much affected by the climate though it was generally believed to be high in the tropics. In view of the common belief that the tropical climate tended to produce an increase in blood sugar, it was thought that diabetes was more common in the tropics. Studies have shown, however, that diabetes is more common in the temperate climes. Serum calcium is appreciably increased probably as a result of excessive production of vitamin D from ergosterol due to the radiant energy of the tropical sun.

(4) Effects on the Nervous System

That hot environment actually disturbs the psychological reactions of the individual migrating to the tropics is generally admitted, but it is doubtful if climate alone is responsible. The climatic element along with a large number of other factors—social hygiene, isolation, sedentary habits,, alcohol, etc.—undoubtedly plays a part. There is, however, a physiological basis for the concept that the higher centres may be influenced by a series of stimuli from the peripheral sense organs and there is a theoretical possibility that the nerve cells in the brain might be exhausted or fatigued if the stimuli are of a type to which the nervous system is not ordinarily accustomed. There are many instances where a close correlation of a pathological nervous state can be made with changes in the climatic and meteorological factors. It is, for example, well known in epileptic colonies that the fit incidence rises when the weather is close and sultry, and before and during storms. Migraine is likewise affected by the weather. Victims of chronic pain have a peculiar interest in the weather. Neuralgia, locomotor ataxia, and other painful diseases are always aggravated when it is damp, cold and windy. The weather may influence the course of chronic psychosis and unfavourable conditions may exaggerate pre-existing states of either depression or excitement.

Irritability.

The intense heat and the bright sunlight of the tropics are the two factors which have been most commonly incriminated as agents in weakening the control of the higher centres over the lower and causing the so-called 'tropical irritability and neurasthenia'. Pediatricians in the tropics have often said that in children, memory, initiative and application become bad after the tenth year. It appears however that the measurable physiological disturbances are on the whole not very significant. Residents in the tropics however, are less prone to feel the depressing effects of a continuous high temperature and humidity, and the nervous effects observed among white settlers are conspicuous by their absence in the natives.

(5) Effects on the Endocrine System and Growth

It has often been observed that the endocrine glands may be disturbed in tropical climes. The association of increased metabolism, high energy level and higher incidence of metabolic diseases in a cooler climate and a sluggish metabolism, a low energy level, decreased resistance to infection and lower incidence of metabolic diseases in a tropical climate are generally recognized, and it is probable that all these factors are ultimately bound up with the question of the activity of the glands of internal secretion.

Thyroid-adrenal apparatus.

Much experimental data, both physiological and pathological, have been collected, indicating that the thyroid-adrenal apparatus may be affected in a hot environment. The defective heat regulation of patients suffering from myxœdema directed the attention of workers to the reaction of thyroidectomized animals to changes in temperature. In the thyroid glands of animals reared in a hot environment definite histological evidence of a diminished colloid content has been found. The activity of this gland is said to be diminished after experimental heat exposure for a certain length of time. So far as the suprarenal gland is concerned, a relationship between this gland and heat regulation has been established by showing that exposure to cold is a powerful stimulus to its functional activity. Definite disturbances of body function due to hypofunction of adrenal glands may result from too prolonged and severe moist heat.

That the thyroid-adrenal apparatus is concerned in the heat regulation of the body is borne out by pathological evidence. Lessions of these glands are frequently accompanied by marked disturbance of heat regulation, and conversely disturbances of heat regulation, as in fevers, frequently produce lesions in these glands. High temperatures are known to affect adrenalectomized animals more easily, and frequently they die in a temperature which is not serious for the control animals exposed for the same length of time. All these facts indicate that there is a lowered resistance to heat in adrenal insufficiency.

These facts enable us to speculate on the close interrelationship that exists between heat regulation and the thyroid-adrenal system and which afford a physiological basis for the study of the problem of the influence of climate on man. It has often been noted that a warm moist climate with little change from day to day is 'relaxing'. This might be the result of a failure to stimulate the thyroid-adrenal apparatus and through it the sympathetic system. Conversely, the cool variable climate, which is associated with increased activity and energy, probably provides a continued stimulus to these endocrine organs and the sympathetic. It is considered that by whipping up the glands of internal secretion (especially the thyroid, adrenals and pancreas) the human organism responds to weather stimulation. The same explanation may be applied to the relation between climate and health. Why is the resistance against certain bacterial infections, such as tuberculosis, increased by a suitably cool, dry climate, and by measures such as open air treatment, hydrotherapy and the like? The answer to the question probably is that those measures are beneficial which stimulate the thyroid-adrenal apparatus without exhausting it and thus strengthen the normal reactions of defence of the organism against bacterial infections. Conversely a warm, moist, monotonous climate weakens resistance against infections, not because it reduces metabolism as many have thought, but on account of the continued absence of stimulus to the thyroid-adrenal and the sympathetic apparatus.

Sexual maturity.

It has often been stated that girls in tropical and subtropical regions menstruate at a comparatively early age. It is also a well-known fact that women marry and bear children earlier in warmer countries than in cooler regions. These facts have led to the belief that the tropical climate induces premature sex maturity. There is, however, no direct proof of this as no reliable statistics are available. In India, various ages, ranging mostly between twelve and thirteen years, have been given for the onset of menstruation. These figures are probably derived from observations on the time of motherhood. The mean age for the beginning of menstruation is 15 to 16 years among the negroes on plantations in Jamaica and Barbados.

(6) Basal Metabolism in the Tropics

Basal metabolism.

The question of the influence of climate on the basal metabolism of the individual is an important subject. No satisfactory evaluation of the effect of the climate on metabolism, however, is possible even to-day, in view of the wide divergency in the results recorded. A review of the work carried out in tropical and subtropical regions in different parts of the world shows discordant results. Some observers have thought that the basal metabolic rate is lower in the tropics. Bose (1934) carried out a large number of observations by the Sanborn technique at the School of Tropical Medicine and came to the conclusion that the basal metabolic rate of healthy normal Indians living mostly on a mixed diet does not differ materially from the accepted standards of Europeans and Americans.

Taking a general view of the whole problem, it would appear that the observations of the majority of the workers who studied metabolism in the tropics are in agreement that there is either a slight decrease or no significant difference in the basal heat production in people living in the tropics as compared with the inhabitants of the cold or temperate regions. It seems inconceivable that such a fundamental factor as basal metabolism should be affected by climate to any great extent any more than the body temperature.

3. Tropical Climate and Resistance to Infection

Bacterial infection.

We have so far considered the effect of tropical climate on the individual involving chemical, physiological and psychical reactions. It would be interesting now to examine whether these changes extend to the bacterial flora of the mucous membranes of the throat, respiratory passages, the gastro-intestinal tract and other parts of the body. It is possible that climatic factors not only influence the physiological forces of the organism but also affect the pabulum on which the bacteria vegetate without in any way affecting the bacteria themselves. This brings us to the question of environment and host resistance, the importance of which from the epidemiological point of view cannot be over-rated. It

seems logical enough to expect, if we accept the evidence that has already been presented regarding the alteration of the chemical, hormonic and psychical status of the individual as a result of environmental changes, the localization of bacteria and other micro-organisms in the tissues is favoured by association with altered membrane permeability, vascular changes, metabolic alterations and ionic shifts. If these changes take place in the particular foci, such as the throat and the mucous membrane of the gastro-intestinal tract, the host parasite relationship may be altered and clinical disorder may result.

Gastro-intestinal tract.

There are many recorded experiments as to the effect of temperature and humidity on the resistance to gastro-intestinal infection. It has been shown that normal dogs exhibit a self-disinfecting power for ingested bacteria. If a living bacterial emulsion was administered by the mouth, these organisms could not be recovered from the cæcum in a viable state. When these dogs were placed in warm rooms (90°—85°F and humidity 90 per cent) and treated in an identical manner, the bacteria ingested by mouth were passed on to the cæcum in a viable state, showing that the heat and humidity caused an interference with the bactericidal power of the dog. Again bacteria injected into the ventrally fixed duodenum of dogs placed in warm rooms soon appeared in the cæcum in large numbers. The same animals, handled in a like manner at ordinary room temperature, destroyed the bacteria before they reached the cæcum. Since in these experiments gastric acidity was excluded, one must assume that there was some other factor besides gastric acidity, or, in other words, that the humid heat caused a change in the bacteriolytic action of the intestinal mucosa. In mice infected *per os* with the same dose of *Salmonella enteritidis* the fatality at a high temperature and high humidity was almost three times what it would be at the same temperature but with low relative humidity.

These experiments show that high temperature associated with high humidity favoured the passage of infecting organisms across the gastro-duodenal barrier and increased the permeability of the intestine so that the bacteria could pass into the lymphatics, and finally reduced the defence of the animal to such an extent that the severity of the infection was enhanced. The fact should not be lost sight of that these experiments dealt with sudden changes of temperature and humidity. Had the experiments been conducted on animals acclimatized to a tropical climate, the results might have been different.

Temperature and dysentery.

In attempting to correlate the experimental findings with clinical data, we are confronted with certain difficulties. It is known that enteric infections, dysenteries, diarrhœa, and colitis increase during the spring and summer months and diminish during the winter. This increase, which is often sharp and sudden, is partly ascribable to the sudden rise in the external temperature which probably inhibits the gastric secretions and allows either infectious organisms or irritating toxic substances to pass more readily into the lower intestinal tract. Furthermore, it would appear that at a high humid temperature the intestinal tract is also more sensitive to the irritating substances produced by the normal intestinal flora. Mills (1928) in China called attention to gastro-intestinal disturbances, associated with nausea and vomiting, following a sudden elevation in temperature and humidity. Similar effects have often been observed in India and Egypt. A sudden change to a high, humid temperature may therefore facilitate infection, or give rise to non-specific irritation. It is possible that there are certain other factors such as seasonal changes in the vitamin content of foodstuffs which are responsible for the rise and fall of resistance.

The character of the intestinal flora of normal individuals during different months of the year under the influence of the usual diet has been studied. The number of bacteria excreted by a normal person was variable. During winter, the number of bacteria was relatively high, and cocci and anærobic spore formers predominated, while in spring there was a transformation with a fall in the bacterial content with the coli group preponderating and cocci and spore-formers diminishing. This indicates that, associated with the sudden rise in temperature, there are changes in the intestinal tract favouring the development of saprophytic and pathogenic members of the colon-typhoid group.

Respiratory tract. infection

Evidence of significant changes in the physiological condition of the nose and nasopharynx in response to changes in temperature and humidity has been produced and it may well be that some of the changes are associated with a lowering of local resistance and a predisposition to cold and other respiratory infections. Cock and Mills (1932) investigated the effect of atmospheric conditions on the upper respiratory tract and found evidence to indicate that it was not only the bacteria which were responsible for the catarrhal inflammation in the respiratory tract, but environmental factors such as seasonal alterations in temperature and humidity were also important accessory factors. External temperature and humidity were important controlling factors in the incidence and death rate of lobar pneumonia. In diseases such as asthma, many workers in the field have

recognized the influence of climatic variations and have more than once shown the association of climatic fluctuation with asthmatic attacks. Evidence has also accumulated which show the seasonal fluctuations of stereptococci in the throat and the severity of attacks of rheumatism and rheumatic arthritis. More information however is needed to prove direct correlation between environmental features and naturally occurring infections of the respiratory passages.

Radiations and resistance.

It has been suggested that ultra-violet irradiation of the body, which is naturally obtainable in the tropics, has the power of increasing resistance to infections. A number of workers have demonstrated a temporary increase in the bactericidal power of the blood as the result of exposure to ultra-violet rays. This view is, however, not universally accepted. A careful study of the frequency of colds among irradiated and unirradiated groups of volunteers at Johns Hopkins Hospital has definitely shown that resistance to infection is not altered by irradiation.

Immunity reactions.

It is not proposed here to enter into the field of immunity reactions and immune bodies in relationship to climatic changes. It is certain that the titre of immune bodies will change with the alterations in the physico-chemical status of the blood. The Report of the New York State Commission on Ventilation (1923) states that rabbits maintained at a temperature of 86°F (30°C) showed distinctly delayed formation of hæmolysins and reduced agglutinating power, as compared with control animals kept at 68°F. (20°C). It has been recently shown that in rabbits, heated so that their body temperature is raised to above 104°F (40°C), agglutinins and bacteriolytic substances are produced in greater quantities than in control animals kept at room temperature.

Allergic states.

A few words may be said about hypersensitiveness and allergy in the tropics as the phenomenon is closely associated with resistance to infection. The Indian tribes of America suffered much less from allergic diseases and were much less susceptible to experimental serum disease than the white races. Acton and Dharmendra (1933) pointed out the rarity of asthma of allergic origin amongst Indians. The reason for this lower incidence is not known and will remain unanswered until the true nature of allergy is known. There is evidence, both experimental and clinical, that hypo-adrenia plays an important role in the production of allergy. It has also been claimed that slight changes in the biochemical make-up of the body might give rise to profound changes in the reactions to foreign bodies and external stimuli. It has been shown, for example, that a shift of the hydrogen-ion concentration to the alkaline side greatly increases sensitiveness in certain tissues. We have already indicated that hypo-adrenia might well be brought about as a result of tropical heat. If this hypothesis is accepted, a higher incidence of allergy in the tropics would be expected.

4. Climate, Health and Disease

Climate and Disease.

The climate of any country, can and usually does, exert a profound influence on its inhabitants. The climatic factors often have a direct bearing on the health. To begin with, the climate of a place very largely determines the food supply available to the people, and this not only affects the general health and well being of the people, but may also result in the prevalence of certain type of nutritional diseases *e.g.,* beri-beri in the rice growing regions, and pellagra in the maize growing areas. Certain types of climates favour the prevalence of particular types of insect life which may be responsible for the spread of insect bourne diseases such as malaria, yellow fever, sleeping sickness, etc. Bacteria are usually very sensitive to climatic conditions and the spread of bacterial diseases is commonly influenced by climatic conditions. For instance it has been established that an intimate relationship exists between temperature and moisture and the spread of cholera, particularly during epidemics. Some diseases occurs in a more intense form in tropical climate than in the temperate one *e.g.,* dysenteries; others occur in a milder form.

Diseases such as rickets are common in northern countries where the ultraviolet rays of sun light are not so plentiful, but are rare in tropical countries. In the tropics, on the other hand cases of heat-stroke are common. It has also been observed that diseases such as syphilis and gonorrhea run a very much milder course in tropical countries. The neurological manifestations of syphilis

which are so crippling, are frequent in the temperate regions but are rarely seen in India. Complications of gonorrhea such as urethral strictures, arthritis etc. are comparatively much less frequent in the tropics.

For a new comer into tropics, temperature of from 20-30°F above that experienced in temperate zones is in itself a considerable strain. Individuals vary a good deal in their reaction to climate, and in every case the reactions produced in most cases are of a temporary nature. The process by which man (or even animals and plants) adopt themselves to climates different to what they are used to is termed acclimatization. In the period during which this transition is taking every effort should be made to lead a careful life and excesses of every kind; dietary exposure to sun etc. should be avoided.

Sunlight.

Sunlight in the tropics is usually very intense, but apart from this it has no mysterious evil properties. Special protection against the heat of the sun should be provided in the form of a proper headgear. In India pugree is worn but a pith helmet is better as it protects the nape of neck and the eyes. Best of all is an umbrella which not only protects the head but a large part of the body as well. During very hot spells, the sun ought to be avoided as much as possible, but otherwise exposure to the sun specially in the cool morning period is beneficial. New comers into the tropics should gradually accustom themselves to the climate and should be particularly careful with regard to exposure to the sun in the begining, acclimatisation takes some time to develop.

Clothing in the tropics should preferably be white in colour, as this absorbs the least of radiant heat. The next best is pale-blue, or a light Khaki colour. Black colours are the worst as they absorb heat. The clothing worne should be loose textured to allow of free circulation of air, and loose fitting. An open necked shirt and shorts, or loose fitting trousers are quite satisfactory as are the shirt and dhoti, shirt and pyjama.

Protection of the head and the spine is important especially if the individual has to be exposed to the direct rays of the sun in the course of his work. For this purpose a hat with wide brims and a spine pad are desirable protections. Lining of these with alluminium foil has been suggested. Dark glasses should be worn to protect the eyes from glare.

Attacks of diarrhœa may be caused by local chilling of the abdomen at night in summer, when sudden unexpected drops in temperature may occur. A light woolen blanket or shawl should be wrapped round the trunk and abdomen especially in persons who have suffered from dysentery or other abdominal conditions. Sitting under a fan after sweating should be avoided.

Housing in the tropics.

In those areas where intense dry heat prevails during summer, the ceilings of the rooms should be high and insulated if possible by being double, with a free ventilation space between the two layers. The walls may also advantageously be built double with a ventilation space in the middle. They should be shaded from the direct sun by a wide verandah. Thatched roofs are cooler than most other types but they harbour insects, vermin etc. In single storied houses a very low plinth is desirable as it utilizes the cool reservoir of the ground to keep the floor cool. In two-storied buildings the lower story is much cooler during the daytime but the upper story is cooler and more airy by night. In every hot weather, it is best to close all the doors and windows during the day, but these should be opened wide in the evening.

In damp climates where the temperatures never rise very high, houses should be built to provide the maximum amount of through ventilation. Insulation of

the ceilings though not so important as in the drier climates is nevertheless desirable.

Air cooling in the tropics.

Under-ground rooms have long been used in places with hot dry summers and very cold winters. The earth remains comparatively cool during the summer and helps to keep the air in such rooms cool and comfortable. Cooling methods dependant on the evaporation of moisture, such as the Khus-Khus screens are placed in doorways facing the prevailing wind and are kept moist, either with a spray or from an overhead tank dripping water. A more modern version of this type consists of a large box which can be fitted into a doorway or a window. On the outer side of the box is a khus-khus screen, kept moist by a drip from an overhead tank, and on the inner side fitted into a circular opening is an electric exhaust fan, which sucks air from outside through the khus-khus screen and discharges it into the room. Such an arrangement is remarkably effective and is very cheap to build and operate. Such a system can be very economically and effectively adopted for use in hospital wards, school dormitories and the like. Besides providing the cooling it would have the further advantage of providing a clean dust-free and insect proof atmosphere. Invalids can also be comfortably taken by trains in the hottest weather by placing a cradle, which is covered by a thick towel kept moist. The patient is kept within the cradle with only the head exposed and an electric fan is turned on to the cradle.

Air conditioning.

Great progress has been made in recent years in applying modern refrigeration methods to the air-conditioning of living rooms, offices, operating theaters, laboratories, factories, etc., and a large variety of suitable plants are available. These range from the small cabinet type of air-conditioners which work off the ordinary household electric supply, to the large conditioning units for factories with many acres of floor space. These installations naturally involve heavy initial expenditure but their value in increasing the working efficiency and health of the inmates has been amply proved, and air-conditioning of factories, hospitals, laboratories and schools is rapidly becoming an accepted requisite. However, where financial considerations do not permit these expensive installations, there is still a great scope for extensive employment of the cheap and simpler devices based on moisture evaporation. Artificially created cool atmospheric conditions will help to maintain better health and more efficiency from point of view of work. Exaggerated fear of catching a chill when going in and out of air condition rooms is not justified. The human body has a very large power of adoptation and when suddenly exposed to heat or cold, the compensating mechanism is atonce set into motion and equilibrium is rapidly established. The writer used an air-conditioned room in Calcutta for many years and never suffered from sudden change of temperature when entering or coming out of the room.

Dietary.

The question of diet in the tropics has been fully discussed elsewhere. In cold climates more food is required inorder to produce body heat. In warm climates less food is required and certain articles of diet, such as proteins and fats particularly should be cut down.

Tellural Influence

Greval (1946) has described the telluric influence on disease and points out that the Indian soil is inimical to certain diseases. According to him the physico-chemical influence of the soil (under the tropical sun) is adverse to the pathogenesis of these diseases is tellural.

Diseases which do not thrive in India. The list of European diseases affected tellurally in India is a long one. The chief amongst them which have attracted attention are:—

Venereal diseases.

(1) Venereal diseases. They do not play the same havoc with the human body in India as in Europe. The incidence of parenchymatous syphilis in India is negligible; GPI is

a rarity against 500 to 700 cases treated yearly since 1927 in England. Tabes is equally rare. Other manifestations of syphilis are mild and yield to simple medication. True WR positive rate for large towns like Calcutta is under 5.3 per cent and in the country must be considerably lower. Gonorrhœa is also mild and does not lead to strictures, crippling, impotence and sterility to the same extent as in Europe. (2) Disseminated sclerosis. This is one of the commonest organic affections of the nervous system in England. In India a typical case again is a rarity. (3) Infantile paralysis. Acute cases seldom attract attention. Badly crippled subjects are seen but rarely. Atrophy in small groups of muscles is detected occasionally in examining recruits for the army. Iron lungs obtained free by some hospitals in India have been used mostly for cases of opium poisoning. (4) Encephalitis lethargica. It hardly ever occurs in its typical form. *Jhinjhinia* of Bengal of 1936 (probably an epidemic of encephalitis) does not leave much damage behind. (5) Other encephalitis of infectious fevers and vaccination. They are almost unknown. (6) Streptocoocal infection. Scarlet fever is rare. Erysipelas is also rare. Subacute infective endocarditis is seen occasionally and proved by repeated cultures until the patient recovers. (7) Leukæmias. Cases are few and far between. Those of the splenomedullary type keep on reporting for examination for years. Some go to Europe for treatment and die within a few months. (8) Kidney disease. Sufferers passing almost pure water not only live but earn their livelihood. (9) Diabetes. Even before the days of insulin Indians retired from Government services because of sugar in the urine and even neuritis lived in extra comfort for many years by finding other employments and adding salaries to their pensions. (10) Kidney disease.

Nervous diseases.

Other diseases.

Recently the unsuccessful search for cases of erythroblastosis foetalis in the maternity hospitals of Calcutta (civil population of the order of 3 millions; so far only one presumptive case reported in this issue has been found) has revealed that even the Rh incompatibility does not operate on Indian soil although the Rh-population exists.

5. Tropical Neurasthenia

The term neurasthenia has been defined in various ways by different neurologists and psychiatrists. This condition is considered by some to be a pathological weakness without discoverable lesion, manifested by rapid and great fatigue, physical or mental, or sometimes both. It is characterised by functional exhaustion of the tissues especially those of the nervous system due to excessive or undue waste of nervous energy, psychic or motor, and in some cases to acute intoxication. The condition, no doubt, is essentially one of emotional unbalance with loss of mental stability, associated with undue irritability to external impulses. Neurasthenics always experience a sense of fatigue or muscular weakness and complain of being unable to do mental labour, the least concentration being followed by vertigo, headache, etc.

This readiness of fatigue is said to be the primary and fundamental symptom of neurasthenia. Beard in his treatise on "The Nature and Diagnosis of Neurasthenia" brings out the literal meaning of the term as a state of nervous exhaustion and he divides the condition into definite separate clinical groups as cerebral, spinal, gastric, cardiac and sexual. It is generally held that such a condition of neurasthenia does never exist, but nevertheless a great variety of conditions in the tropics where any specific cause cannot be discovered is termed as an idiopathic disease 'neurasthenia.' The modern idea however is that there is some underlying factor causing the train of symptoms constituting neurasthenia. Investigation into the subject reveals that neurasthenia is associated with certain existing causes. Tropical neurasthenia responds to treatment more readily than the ordinary kind, change of climate being especially beneficial.

Meaning of term.

Neurasthenia was for a long time attributed solely to exhaustion of the nerve centres presiding over general nutrition and particularly of the brain and nervous system. Actual loss of substance in the cells and especially in the nucleus has been noted by many workers. Impaired metabolism with accumulation of waste products in the system gives rise to auto-intoxication affecting especially the nervous system and the functions over which this system presides are correspondingly impaired. In this condition if the patient persists in imposing upon his weakened organism even slight tasks, a vicious circle of pathogenic activity is formed which encourages a further waste of energy.

Left to itself neurasthenia tends to persist, unless its cause is removed. Under proper prophylactic measures and judicious treatment the prognosis is favourable, especially in the cases where organic changes have not had time to undermine the functions of the organs secondarily involved.

(1) Aetiology and Incidence in the Tropics

From a study of the causes of neurasthenia, the condition has been well classified as:

Primary.

I. Primary or independent of any discoverable exciting causes; this has been termed 'congenital' or prenatal. Heredity acts only as a predisposing influence through parental neurosis or psychoses. No gross visceral damage, nor any evidence of serious illness or infection is met with in this type. The signs and symptoms are chronic in nature or a recurrent one. A careful history will reveal that the individual belongs to a stock of similar victims and this is essentially one of a familial nature. This type of neurasthenia is very common in the tropics and mostly met with among children in the same family. They manifest it more and more with age, at a period of life during which great exertion and anxiety combine to increase the wear and tear of the central nervous system and indirectly of the organism at large. Climatic influence in the tropics and the faulty up-bringing of children in such families account for the development of such a state of mind and brain in them. They can carry on the ordinary routine of life but any slight deviation from it will surely throw them out of gear resulting in signs and symptoms of nervous exhaustion. The picture may be well drawn of such a neurasthenic as suffering from various bodily pains (paræsthesias), abnormal sensations of the brain, heart and stomach (hypochondria). The appetite is capricious, ravenous one day and bad the next day. In short, the whole condition is one of 'irritable weakness with hypochondrial trend.'

The underlying psychopathology of these primary neurasthenics is that the cell units and the neurokyme in them are not sufficiently strong to keep in check and in store the potential energy responsible for the normal body functions. A continuous drainage of such energy demanded by an increased body activity, with which the inherently defective neurones cannot successfully keep pace, throws the whole system out of balance resulting in a condition of neurasthenia.

Secondary.

II. The second group constitutes a larger number of ætiological factors and they all come under the type of secondary neurasthenia, secondary to some primary causes such as physical ill-health with lowered vitality, condition of life incompatible with environment, overwork, emotional stress. It is rightly said that it is not the work but the worry that kills. Sudden physical and mental shock, continued conditions of fear as seen in maniacs when they are not actually suffering from any disease, sometimes predispose to an attack of neurasthenia.

Besides the factors mentioned there are others met with. People in the tropics live in a climate in which humidity is added to heat and this will act as a primary causative factor. There are diseases also which are peculiar to the tropics and are seldom seen elsewhere. All these affect the daily lives of the people in a way different to the people of temperate zones. The maladies and the physical agents such as extreme changes of weather, occurring in tropical countries are responsible for the chronic ill-health of people and should be considered as factors bringing about a series of signs and symptoms constituting the so-called condition of neurasthenia. Such causes may be conveniently classified as (*a*) exciting, (*b*) predisposing, and (*c*) general endocrine dysfunction.

Aetiology.

(*a*) *Exciting causes.* The debilitating after-effects of acute fevers in the tropics, exposure, indiscretions in diet, insufficient or improperly selected food , etc., are potent factors of after-neurasthenic symptoms. All convalescents after acute maladies either promptly recover without after-effects or may suffer from chronic ill health for a long time with symptoms of neurasthenia. Various forms of enteric fevers, dysentery, cholera, intestinal helminthiasis, etc., give rise to disturbances of the gastro-intestinal functions. The continuous absorption of toxins elaborated lowers the resistance of the organism as a living entity (not only of the nervous system), undermines the metabolic dynamism and prepares the soil for neurasthenia.

Individuals so predisposed represent by far the majority of cases. Women suffer almost invariably with uterine diseases in connection with neurasthenia. Excessive fecundity, dysmenorrhœa and menopause are thought to exercise a marked exciting influence. Disorders of the female organs which affect the nutrition of the nervous system, such as an excessive hæmorrhage or suppurative process, may also be important factors in inducing functional neuroses.

Of all the causes those connected with the male sexual organs have been credited with the most active ætiological role such as prostatitis, posterior urethritis, seminal vesiculitis, etc., and general disorders and habits such as gonorrhœa, syphilis and masturbation.

(*b*) *Predisposing factors.* A continued high atmospheric temperature with excess of humidity makes people in the tropics ease-loving and much less hardy than those of the

temperate climes. A little superadded physical or mental strain often brings about a nervous breakdown and makes a man physically unfit to cope with his environment. People working in the open spaces get easily tired owing to heat of the sun and cases are on record where many a people after long continued illnesses as malaria, kala-azar, etc., and apparently healthy, have succumbed to heatstroke.

Due to climatic influence in the tropics and to keep body temperature below the surroundings a large quantity of blood is regularly required to flow in the peripheral circulation especially in hot weather. People lose appetite in the hot weather, metabolism and ordinary physiological functions and biological processes are depressed lowering body vitality and making them an easy prey to nerve exhaustion. Again in hot weather, people are prone to remain indoors to protect themselves from hot blasts of air. The resulting ill ventilation with all its effects tells upon the health of the people. Anæmia and other diseases of the blood due to improper aeration of the blood-cells result. People use nets to prevent the biting of disease-producing mosquitoes and this is considered to be sleeping under unhygienic conditions leading to general depression of body vitality.

The social conditions of people living in the tropical countries contribute a great deal to the causation of symptoms of neurasthenia:—

Contributing factors in Tropics.

(1) *Heredity.* It accounts for a good number of cases. This has already been dealt with in discussing primary or congenital neurasthenia. Women are more prone to this type. If, as maintained by sexologists, marriage is an important factor to prevent neurasthenic symptoms in grown up unmarried girls, definite cases are on record where married, multiparous women suffer from neurasthenia without any other obvious discoverable causes. Other social conditions as early marriage, early and repeated pregnancies associated with ill-nutrition and strain and stress of life and other maladies make people of the tropics frequent subjects of neurasthenia.

(2) *Diet.* People in tropical climates live on a diet which can be hardly borne by those of temperate countries and so it is held by authorities of public health that such a quality of diet cooked with highly-spiced articles, mustard oil with its irritating allyl isothiocyanate, etc., is responsible for gastric troubles such as hyperchlorhydria, gastritis, peptic ulcers, etc. The digestion is enfeebled and delayed and is associated with atonic constipation and gastralgia is sometimes complained of. Hyperchlorhydria is sometimes observed and gastroptosis is not infrequent. Meteorism, alternating constipation and diarrhœa, colicky pains due to defective intestinal digestion and the resulting fermentation are prominent features of the later stage. In these cases, auto-intoxication is an important feature.

(3) *Exercise.* That diet and exercise should go hand in hand is an old adage. Fat and forty-year-old Indians are often diabetics. Visceroptosis and cases of prolapse-uteri in quite young Indian girls are the result of lack of proper exercise and such girls always suffer from vague symptoms of neurasthenia such as indigestion, attacks of abdominal pain (hypochondria) associated with various endocrine dysfunctions and particularly of the thyroid and adrenals. All these predisposing causes combined with toxins of acute and chronic illnesses characteristic of the tropics play an important role in the causation of neurasthenia.

(*c*) *General endocrine dysfunction.* The endocrine system may be thrown out of order, as a result of the patient suffering from some acute disease such as enteric fever, cholera, bacillary dysentery, small-pox, etc. Long-continued illness such as malaria, kala-azar, chronic genito-urinary diseases or absorption of the toxins arising from putrefactive changes in the gut may occasionally put an undue stress on the glands such as the thyroid and adrenals. That the ductless glands play an important role in the genesis of neurasthenia is probable. The thyroid and adrenals play an important part in oxidation and in protecting the body against auto-intoxication, and any condition which exhausts these organs must necessarily impair the general dynamism and lower the vascular tension, the underlying cause of neurasthenia in many cases. It must be clearly understood in this connection that cases classified formerly as neurasthenia are due to disorder of the internal secretions. In dysthyroidism there are recurrent states of neurasthenia and great prostration. Pituitary disorders too create symptoms of neurasthenia. Insufficiency of the adrenals also produces neurasthenia characterised by extreme asthenia, inability to think clearly, and as a rule associated with low blood pressure.

Diagnosis.

There is practically always in true neurasthenia a long prodromal period, the pre-neurasthenic state, characterised by well-defined morbid manifestations indicative of aberrant functioning of the emotional centres. The immediate cause of this instability is the reaction of environment on the patient's mind. This state is not actual neurasthenia and may not develop into it, but neurasthenia is its culminating development. Neurasthenia is a definite syndrome and should not be confounded with the nervous asthenia resulting from abuse

of stimulants, drugs or tobacco as being symptomatic of organic diseases. Neurasthenia should be clearly differentiated from obsessions, hysteria, melancholia and hypochondriasis. The heart and blood vessels are usually the first to reflect the central functional disturbance in neurasthenia.

Reviewing the innumerable factors responsible for bringing about the condition of neurasthenia, there is no doubt that it is a malady not without obvious causes. A proper diagnosis should be made, largely by a process of exclusion. All possible gross organic diseases are to be excluded and careful investigation is most essential regarding the daily life of the neurasthenic together with complete physical examination of the subject. If this procedure is not adopted several diseases of the body and mind will be wrongly diagnosed as neurasthenia. Amongst them early phthisis with symptoms of indigestion, feeling of ill-being in an apparently healthy young man, diabetes, Addison's disease, disseminated sclerosis, cerebral tumour, cerebral arteriosclerosis and encephalitis lethargica, general paralysis of the insane and dementia præcox deserve special mention.

A line of demarcation should be drawn between real organic diseases and the functional disorders of the nervous system. Besides the diseases mentioned which simulate symptoms of neurasthenia there are many more which cannot be discussed here for want of space.

(2) Treatment

General.

The prognosis and the line of treatment depend chiefly on a right diagnosis of the cause of neurasthenia. Cases of secondary neurasthenia always make a complete recovery after removal of the primary causes and all that is needed is proper rest and treatment of those diseases. There may occur periods of improvement but relapses occur readily.

The treatment of cases of neurasthenia does not merely consist in the application of specific drugs against such diseases as are responsible for its development. Although the importance of such a procedure should always be recognised, general tonics such as phosphorus (glycerophosphate of iron, both organic and inorganic), arsenic (with all its synthetic preparations) and strychnine combined with nourishing and proper dieting, regular and graduated exercise and open-air life, rest mental and physical, distraction and removal of baneful influences as far as possible constitute the prominent features of the treatment.

The benefits of rest in the average case may be secured by spending four to six additional hours in bed, by retiring early and getting up late. Or, if it is convenient, a couple of hours' rest during the day may replace the morning hours of rest. Each phase of the non-pharmaceutical treatment must be regulated to suit the patient's means and strength. Travelling is always useful, changes of scene greatly tend to alter the morbid trend of the mind.

Isolation is beneficial when neurasthenia is accompanied by very marked symptoms of lowered nutrition and muscular weakness and when a prolonged rest in bed is insufficient to arrest emaciation. Over-feeding is sometimes obligatory and may be carried out by the addition of milk and eggs to an ordinary mixed diet between meals.

Gastric functions should be analysed and diet adjusted accordingly along with administration of dilute hydrochloric acid before meals where hypo- or anacidity is found. Any foci of infection present should be properly dealt with.

Women mostly suffer with a train of abdominal symptoms due to lack of tone of involuntary muscles of the various abdominal organs and such diseases are cœcal stasis, prolapse of abdominal viscera and various other pelvic organic diseases. Patients with visceroptosis are very commonly met with complaining of vague abdominal pains. A skiagram always settles the diagnosis. The patient should be advised proper abdominal exercises, small but frequent dieting, a well-fitting abdominal belt and sometimes electrial treatment with sinusoidal

current. Cases are on record which show that this line of treatment has cured many such neurasthenic patients. Chronic cœcal stasis should be treated by regular abdominal massage and by drugs such as cascara, senna leaves or pods stewed with prunes, etc. Sometimes surgical measures are adopted to cure patients with neurasthenia. Removal of the appendix, fixation of the floating kidney, gastropexy and a few pelvic operations in females have been known to cure neurasthenic patients.

In cases of neurasthenia, resulting from sexual abuse, rest of function is essential. Local treatment, especially of the verumontanum has been widely advocated, but some believe that such treatment only acts by suggestion. Sexual neurasthenia is often ascribed to a definite pathological condition of the genito-urinary tract especially in the vicinity of the verumontanum but some authorities do not agree on the point. Electricity has been advocated by many. Static electricity, slowly interrupted faradic current or diathermy may be conveniently employed.

Hydrotherapy has also been highly recommended. Winternitz's method of cold pack along with the application of warmth (122°F. or 50°C.) over the epigastrium or the application of cold over the spine is credited with marked efficacy. Sleep is favoured by taking a warm bath for ten minutes followed by a glass of hot milk just before retiring.

Drugs.

Medicines. Few medicines are of value; the elaborate process of nutritional repair, favoured by the aid of the above-mentioned procedures, renders drugs relatively unnecessary.

Neurasthenia is recognised to be a vasomotor neurosis, the prominent feature of which is relaxation of all arteries, due to exhaustion of the sympathetic centre and the resulting loss of propulsive power of the arterioles. The tissues thus become imperfectly oxygenated and nourished, hence mental torpor, habitual fatigue, adynamia and gastro-intestinal atony result. There are various functional disturbances associated but certain degree of hypothyroidism is always present. Hence small doses of desiccated thyroid is recommended by some, usually in combination with strychnine, and full amounts of an assimilable form of iron such as Blaud's pill. Strychnine is supposed to be almost a specific in neurasthenia. Doses should be gradually increased and excess of 6 mg. (1/10 gr,) may be given in daily doses. Arsenic, iron and other tonics are often valuable. Of the glandular products, thyroid gland (Armour's) in 1 gr doses (which equals 5 gm of fresh gland) or pituitary (whole) 2 gr three times a day are very efficient. Corpus luteum in large doses is sometimes very effective in women.

Laxatives are important to counteract the auto-intoxication. The intestine should be flushed with calomel and a saline purgative at the start. Later, cascara, rhubarb, or aloin are suitable. The endless complaints and fault-finding nature of neurasthenics are in most cases symptomatic. Psychotherapy is usually effective in such cases. Sympathy and consideration on the part of the attending physicians, always gain the patient's confidence and ensure his co-operation which is of primary importance in the curative measures instituted.

Under proper prophylactic measures and judicious treatment however, prognosis is usually favourable. The just merit of each individual case should be taken as the guide to treatment.

CHAPTER XII

METABOLIC DISEASES

DIABETES MELLITUS: AETIOLOGY AND CLINICAL ASPECTS; TREATMENT: DIETETIC, INSULIN—HYPOGLYCAEMIA AND DIABETIC COMA—OBESITY: AETIOLOGY AND CLINICAL ASPECTS, TREATMENT—INFANTILE BILIARY CIRRHOSIS—GOUT.

DIABETES MELLITUS

Diabetes mellitus is a disorder of metabolism, characterised by polyuria, excessive thirst, hyperglycaemia and glycosuria, and a progressive loss of weight.

Historical.

Diabetes is a disease which should prove to be of great interest to any one working in India not only because it is extremely common here, more particularly in Bengal, but also because the disease was known to the ancient Hindu writers as early as the 6th century B.C. Charaka (2nd century A.D.), the most renowned Hindu physician of his time, described some of the cardinal symptoms of diabetes (including the presence of sugar in the urine) in his Charaka Samhita. It appears that Charaka collected his materials from a much earlier work of Agnivesa who again based his writings upon the teachings of his master Atreya (6th century B.C.). It is a disease which may be said to have its home in the tropics. What gout is to the nobility of England, diabetes is to the aristocracy of this country. Among the ancient Greek writers, the symptomatology and prognosis of diabetes are to be found in the writings of Celsus (25 A.D.). The name diabetes (which means 'to pour through a syphon') was given by Arateus in 30 A.D. Among the European writers Thomas Willis, an English physician of repute, first noticed the characteristic sweet taste of diabetic urines in 1670, thus establishing the basic principle for diagnosis between diabetes mellitus and diabetes insipidus.

1. Aetiology and Clinical Aspects

Aetiology.

In 1889, Von Mehring and Minowski produced diabetes mellitus experimentally in dogs by extirpation of the pancreas. Later, Opie showed that the pathology of the disease was centered in the islets of Langerhans; and in 1922 Banting and Best succeeded in preparing insulin from the islet tissue of glands in which ligation of the pancreatic duct had produced degeneration of the acini. Signs of chronic hyperinsulinism have been observed in a number of cases, in the presence of adenomata of the islet cells.

The consensus of the present day opinion is that a pancreatic lesion plays the most important part in the aetiology of diabetes and that the root cause of diabetes centres round the islands of Langerhans though the nature of the factor or factors giving rise to the diseased condition of the islet cells has not yet been clearly understood. The predisposing causes which directly or indirectly are likely to throw undue strain on the islet cells of the pancreas and cause their hyaline degneration and atrophy sooner or later are many, the more important among them being sedentary habits, lack of physical exercise, ill balanced diet, overeating, too much mental work, infections, etc. Heredity is also believed to be an important factor. In some of the writer's series of cases, diabetes could be traced to run for three or more generations.

Climate is also believed by many to be one of the factors. It has been stated that tropical and sub-tropical climates lower the carbohydrate tolerance of individuals. Basset-Smith has observed that under the influence of the tropical heat, there is a tendency to the retention of carbonic acid in the alveolar air in people at rest and also to the production of glycosuria and acidosis particularly in the well fed.

Coming to the incidence of diabetes among the different races of India the writer is of opinion that Bengalee Hindus appear to be more prone to diabetes than any of the other races, the proportion being 68 per cent among the total cases.

Infections of any kind may prove to be a direct cause of diabetes, probably owing to some kind of damaging effect on the pancreas by the toxin. Lawrence and Buckley have proved by experiments that diphtheria toxin will practically annihilate the action of insulin.

Pathology.

The commonest lesion found in diabetes mellitus, is a hydropic degeneration and vacuolation of the beta cells of the islets of Langerhans. These changes are however absent

in many cases, particularly in children, in whom it has been suggested that the cause is a congenital reduction in insulin forming tissue, rather than the result of a pathological process.

Symptoms.

Clinically two principal forms of diabetes are recognised, acute and chronic. The acute form is generally found among young people and usually runs a rapid course. Mild chronic forms of the disease are usually common in middle aged persons but such cases may gradually develop into the severer form and then the disease runs the usual rapid course.

Mild cases may be entirely without symptoms, or these may be confined to a mild degree of polyuria or a feeling of fatigue at the end of the day. This type is most frequently encountered among elderly people who are often obese and have arteriosclerosis. In severer cases, thirst, polyuria, excessive fatigue and loss of weight are usually present. Pruritis is common, especially amongst women. Visual disturbances are frequently complained of, and cataract may develop. Excessive hunger is often met with in young diabetics.

A condition of mild neuritis is often met with and causes pains in the legs, diminished vibration sense, and loss of ankle-jerks. Severe neuritis is uncommon.

Diagnosis.

The diagnosis of true diabetes mellitus is often an easy matter when the patient presents the usual signs and symptoms; but there are many cases in which considerable difficulty is experienced and elaborate laboratory methods of diagnosis are necessary.

The average normal fasting level of blood-sugar in man is 0.1 per cent. After a meal consisting of carbohydrates, the blood sugar rises reaching a maximum level of 0.17 per cent within one hour and coming down to the normal level again, usually within 1½ to 2 hours. The reason why it comes back to the normal level is because a part of the extra sugar is changed into glycogen and stored as such in the liver and part of it undergoes combustion in the muscle tissues. In the diseased condition, the picture is quite different for owing to the inability of the diabetic individual to store sugar as glycogen or burn it like a normal person, the circulating blood of the diabetics contains excess of glucose. Thus in mild cases, the fasting level is usually found to be 0.13 per cent or over. In more severe cases, it is of course proportionately higher, according to varying degrees of defect in the storage mechanism.

In many cases of potential diabetes and even in cases of mild diabetes, however, the fasting level of blood sugar may be perfectly normal and the urine may be aglycosuric even after meals owing to a varying leak point. In such doubtful cases, the glucose tolerance test should be done and the behaviour of the blood sugar after a glucose meal should be investigated. A healthy normal person should conform to the following:—

(1) The fasting blood-sugar level should be normal.

(2) The maximum rise of blood sugar after ingestion of 50 gm of glucose should take place within one hour and should not go beyond 0.17 per cent.

(3) The drop of the blood-sugar to the normal level should take place within 1½ hours after the glucose is taken.

(4) No glycosuria should occur.

A diabetic blood-sugar curve after a glucose meal deviates from the above according to the varying degree of defect in the storage mechanism, judged according to the rise of the initial (fasting) level of blood-sugar, a tendency to a much heightened curve and a much delayed return to the normal level.

2. Treatment

The fundamental principle underlying the successful treatment of diabetes is based on the knowledge that a careful adjustment of the diet, with or without the use of insulin as an adjunct, will prevent hyperglycaemia and hence eliminate glycosuria and thus will tend to give rest to the already overworked and diseased islet cells of the pancreas. There is plenty of evidence in the literature to prove that if hyperglycaemia can thus be prevented, the progress of the disease will be arrested gradually, the distressing symptoms of the disease will disappear and in the majority of cases the 'tolerance' of the patient will improve considerably.

Thus it appears that the treatment of diabetes mellitus falls mainly into two main groups:—

Dietetic. (1) Dietetic treatment. This means that the patient should be given only that amount of food which is absolutely necessary to meet the minimum metabolic requirements of the body. This is known as the basal diet.

(2) Insulin treatment. It should be remembered that insulin treatment is only a valuable adjunct to the dietetic treatment for which it can, in no sense, be considered to be a substitute. The indication for insulin treatment will be considered later on.

(1) Dietetic Treatment

In calculating a basal diet for diabetic patients the following points should be considered:—

(1) The caloric value of the diet should not be less than 25 calories per kilo of the patients' body weight.

(2) The protein content of the diet for an young adult should be about 14 gramme per kilo of the body weight.

(3) The fat content of the diet should be fairly low.

Bose's formula for calculating the basal diabetic diet is given below:—

Total caloric requirement of the patient = weight of the patient in kilo $\times$ 25

$$\text{Protein requirement in grammes} = \frac{\text{Total caloric requirement}}{20}$$

$$\text{Carbohydrate requirement in grammes} = \frac{\text{Total caloric requirement}}{16}$$

$$\text{Fat requirement in grammes} = \frac{\text{Total caloric requirement}}{16}$$

Thus a diabetic individual weighing 64 kilo (10 stones) will require the following diet:—

Total caloric requirement = $64 \times 25 = 1{,}600$ calories.

$$\text{Protein requirement} = \frac{1600}{20} = 80 \text{ grammes}$$

$$\text{Carbohydrate requirement} = \frac{1600}{16} = 100 \text{ ,,}$$

$$\text{Fat requirement} = \frac{1600}{16} = 100 \text{ ,,}$$

The number of grammes of carbohydrate, protein and fat and the caloric

value of the diet required being thus ascertained, the patient's diet is framed by the use of food tables.

For ready reference, a sample diet chart (suitable for a diabetic patient weighing 64 kilo as mentioned above), having the above proportions of food principles is appended below:—

Bread	5	oz
Butter	1	oz
Eggs	2	only
Fish or chicken	4	oz
Mutton	6	oz
Green vegetables	16	oz
Milk	8	oz
Ghee	1½	oz

Approximately, Carbohydrate=100 gm; Protein=80 gm; Fat=100 gm; Calories=1600.

On a diet calculated as above, the average diabetic patient of the mild type usually becomes sugar-free in about four days and the blood sugar comes down to the normal level in about a week's time. If however the patient does not become sugar-free or does not maintain the blood sugar at the normal level on the above diet, insulin treatment is usually indicated.

(2) Insulin Treatment

As mentioned before, insulin treatment in cases of diabetes should be considered as a valuable adjunct to the dietetic treatment in order to get the full benefit of the insulin and the quantity of diet allowed. It should also be kept in mind that unlike most drugs, insulin has no fixed dosage and that no hard and fast rule can be laid down regarding the dosage of insulin, inasmuch as it may vary in individual cases, according to the severity of the case, complications, the diet prescribed and various other factors. *Insulin.*

Another important factor which should be considered is that the amount of glucose utilised per unit of insulin varies in different cases. It has however been roughly estimated that one unit of insulin usually causes 1 to 2 gm of carbohydrates to be utilised in a moderately severe case of diabetes. In a milder case, the carbohydrate utilisation per unit of insulin is somewhat greater. This fact should be borne in mind in selecting the proper dose for a given patient.

Roughly speaking, the total amount of available glucose calculated from the patient's diet minus the total amount of glucose passed in the urine will indicate the carbohydrate tolerance of the patient and the amount of glucose which is over the limit of tolerance, *i. e.*, the total amount excreted in the urine must be covered by an adequate dose of insulin calculated in the way mentioned above.

Hints on insulin treatment.

1. There are no hard fast rules regarding the dosage of insulin in a particular case as it varies with the complications and the amount of fasting hyperglycæmia. It is always best to begin with a small preliminary dose.
2. Insulin is certainly indicated in all cases of severe diabetes mellitus with pronounced hyperglycæmia and marked glycosuria with or without ketosis.
3. Insulin is also indicated in the milder forms of the disease where a careful dietetic regime has failed to remove glycosuria or reduce the fasting hyperglycæmia, say below 0.15 per cent.

4. It is always best to give insulin injection, 20 minutes to half an hour before meals.

5. All forms of hard or strenuous exercises should be forbidden for at least 3 to 4 hours after insulin injection.

6. Roughly speaking it may be assumed that one unit of insulin causes 1 to 2 gm of carbohydrates to be utilised in a moderately severe case of diabetes. In milder cases the carbohydrate utilisation per unit of insulin is greater.

Insulin Hypoglycaemia.

Early symptoms (1) Unaccountable nervousness. The patient feels some impending danger. (2) Listlessness. (3) Tremulousness or actual tremors, particularly of the extremeties. (4) Dim vision sometimes diplopia. (5) Great desire for food—a sinking feeling in the pit of the stomach. (6) Vasomotor disturbances—pallor of the face alternating with flushing and sweating. (7) Palpitation of the heart.

Late symptoms. (8) Mental confusion—low muttering delirium. (9) Loss of deep reflexes. (10) Muscular twitchings and convulsions. (11) Coma.

Treatment. The treatment is simple if undertaken early. The patient should be put to bed. A tablespoonful of glucose powder dissolved in water and given by the mouth is the best but if it is not at hand, ordinary table sugar will form quite a good substitute. Orange juice 4 to 8 oz. may also be given, if available. If the symptoms persist after ¼ hour more sugar should be taken. If the milder symptoms are overlooked or not given heed to and they are allowed to pass on to the coma stage, ½ to 1 ccm of solution adrenaline chloride (1 in 1,000) should be given intramuscularly. Glucose may be given intravenously, if necessary.

3. Hypoglycaemia and Diabetic Coma

A diabetic patient may become comatose because he has either too little or too much insulin. Two kinds of coma are generally recognised and the signs and symptoms following one should be distinguished from the other.

In insulin (or hypoglycæmic) coma the normal colour of the skin is generally preserved or it may be very white. The respirations are shallow but the breath does not smell of acetone. The urine is usually sugar-free and does not contain aceto-acetic acid but may contain both if the bladder has not been emptied for some hours. The blood sugar is below 0.07 per cent or may be as low as 0.04 per cent. In diabetic coma on the other hand, the symptoms are fortunately quite characteristic. The skin is usually flushed, the respirations are deep and the breath smells of acetone. The urine always contains large amounts of sugar as well as acetoacetic acid and the blood sugar is over 0.2 per cent and may be even as high as 0.5 to 0.8 per cent. In hyperglycæmic condition, if the patient is not deeply comatosed the initial dose should be 50 units but if coma be deep, 70 or over 100 units of insulin should be injected at a time. But if the patient is used to large doses of insulin, the initial dose should be at least half as great as the doses recommended for ordinary cases. Administration of sugar is always desirable along with the injection of insulin to avoid hypoglycaemic coma. In cases of deep coma, 600 ccm of a 2 per cent solution of sodium bicarbonate should be given with 50 gm of glucose. Along with the insulin and glucose treatment estimation of blood sugar where possible and particularly the testing of urine for sugar every three hours should always be done to note improvement of the patient. A repetition of treatment may be necessary where improvement is slight or even absent. The second course of treatment usually begins six hours after the first one. If the patient is better, half the initial dose of insulin should be given; if no improvement follows the initial treatment, the previous dose

should be repeated; and if the patient is worse, the dose should be half as much again.

The risk of precipitating a hypoglycæmic coma should always be borne in mind while carrying on insulin treatment for diabetic coma. The most usual signs in such cases is a relapse into deep coma after a partial recovery of consciousness. This emergent condition demands prompt treatment. One ccm (ten units) of pituitrin or one ccm of adrenalin should at once be injected and this is to be followed by an intravenous injection of 300 ccm of 20 per cent glucose solution. The treatment raises the blood sugar and the patient regains consciousness. In the following lines a general outline of treatment is given.

1. Put the patient in bed in charge of a trained nurse. Keep him warm with blankets and hot water bottles, if necessary. If there are indications of circulatory collapse, give digitalin, caffeine, camphor or other suitable drugs.

2. Evacuate the large bowel by an enema. If there is much vomiting gastric lavage should be done.

3. Give insulin as soon as possible. No hard and fast rule can however be laid down regarding the initial and the subsequent dosage of insulin and the mode of its administration in as much as it depends on the depth of the coma, the general conditions of the patient, the blood-sugar level and various other factors. As a general rule, it may be said that in a case of moderate severity, an initial dose of 40 to 50 units or more of insulin may be given subcutaneously or intravenously, followed by the same amount of glucose in grammes, administered by mouth if the patient can swallow, or intravenously if the patient is totally unconscious.

4. In severe cases, where there is severe dehydration due to loss of fluid and collapse, prompt treatment is essential to save the patient from death, and the case is to be treated on similar lines as in cholera. Introduction of saline intravenously, subcutaneously and rectally should be done as early as possible. It is best to combine 10 per cent glucose with normal saline. One pint of this solution will contain 60 gm of glucose and should be given very slowly by the intravenous route immediately after the insulin injection. The injection of 0,5 ccm of adrenalin chloride solution (1 in 1,000) in addition sometimes helps in restoring the feeble pulse. This may be repeated after 4 hours according to the state of the collapse. If the patient's condition improves sufficiently, only insulin and glucose should be continued. The dose of insulin will however have to be varied according to the state of the acidosis and hyperglycaemia, judged from the urine and blood-sugar test. The dose of glucose should also vary according to the amount of insulin given. Rectal saline should also be given. Solution of the same strength as used for intravenous injection may be given. Sodium bicarbonate (1 dr. to a pint) may be added to the fluid with advantage; 4 to 6 oz. of this fluid should be introduced per rectum very slowly every 4 hours.

OBESITY

1. Aetiology and Clinical Aspects

Obesity bases as a disease of fat metabolism and is characterized by superfluous deposits of fat in the body.

Aetiologoy.

To define obesity as a disease of overnutrition would, however be more correct. In healthy man the need of food is undeniably registered as hunger and the filling of the need by disappearance of appetite and a feeling of satiety. This regulatory principle suffers under abnormal nutritional circumstances. We learned this fact in Germany in the years following the First World War. Foodstuffs which the Germans had to deny themselves for years were available again in increasing amounts. The mechanism which had been practically out of use for so long no longer functioned accurately. There followed a

reinstated almost physiological hyperappetite. The joy, so long denied, of eating one's fill, kept the voice of satiety from sounding the warning in time or at all. The result was a rapid increase in obesity from 1924 to 1926.

For practical purposes it may be defined as a condition in which there is an increase of about 20 per cent of fat over that found in an individual of average weight. The weight of the person therefore is 20 per cent over the normal for his age and height. The condition of obesity is frequently met with in tropical climates, and particularly among certain classes in India who indulge in foods rich in carbohydrates and fats and take little exercise. The condition runs in families and there appears to be an hereditary tendency towards its production.

The effects of such a condition on the physical and mental processes of an individual are obvious. Fat-laden persons are known to be more liable to microbic infections. An extraordinary degree of impairment of muscular efficiency and signs indicative of cardiac embarrassment can be found in these people. Fatty changes in the heart muscle and abnormal blood pressure are frequent accompaniments. As a general rule, obese persons avoid effort, both mental and physical, and their critical faculties are to a considerable extent dulled. In addition certain other common maladies such as constipation, dyspepsia, osteo-arthritis, etc., are frequently observed in these cases. The effect of obesity in causing sterility in women has been recognised. It has an additional importance from the æsthetic point of view.

It is customary to describe two varieties of obesity:—(1) Exogenous obesity, in which there is a faulty balance between the intake of food and the output of energy, and (2) endogenous obesity which is due to obscure constitutional factors, such as endocrine or metabolic disturbances. In practice, however, it is found that a clean cut distinction cannot be made between the two varieties and many cases of obesity present the features common to both conditions.

Exogenous obesity.

It is almost an axiom that obesity is due to over-eating. In these cases there is a great disparity between the intake of food and the output of energy in the shape of muscular work. Poulton (1931) observed that most people keep the same weight from year to year which means that the amount of food taken is exactly balanced by the amount burnt in the body. Three processes are responsible for this, namely (*i*) the so-called basal metabolism of the individual, (*ii*) specific dynamic action of the foodstuffs, which means that when food is taken its metabolism in the body leads to increased burning, and (*iii*) exercise which increases combustion to supply the extra energy required. Hence to maintain the normal weight there must be a close relationship between the food taken and the activities of the individual. If the quantity of fuel taken as food is much in excess of what the organism requires there will arise a tendency to obesity, and when this is continued for a long time it may result in deposition of considerable fat. Obesity in the adult is in a very large proportion of cases due to over-eating. During childhood and adolescence, the amount of exercise one takes precludes any deposition of fat. As youth merges into middle age, violent exercise is replaced imperceptibly by exertion of a less drastic nature. Hence it naturally follows that when utilization of foodstuffs is no longer so large, the amount of food during the period should be curtailed. If that is not done, an accumulation of fat occurs in many cases.

Certain foodstuffs are particularly liable to cause accumulation of fat in the body. In addition to the use of fat-forming foods, malt liquors are also known for their fattening properties. Intemperance in the use of alcohol is also an important factor, for alcohol is readily oxidized and thus allows the fat already formed to remain undisturbed. A suggestion was put forward by Weber (1928) that exogenous obesity may be the result of pancreatic stimulation following the ingestion of too much carbohydrates.

Endogenous obesity.

Constitutional obesity. While it is possible to attribute corpulence in some persons to dietetic excesses it is well known that certain obese people eat remarkably little and yet have a tendency to accumulate fat. Strouse and others (1924) observed that obesity might occur in certain persons on intakes much below the necessary caloric requirements, the cause of which is imperfectly understood. It is, therefore, believed that this type of obesity is brought about by a qualitative anomaly in metabolism, *i.e.*, an abnormally increased transformation of carbohydrates into fat, and thus even with caloric intake which is much below the normal, such a patient will convert fat from the carbohydrates and store it as such. The part played by heredity in causing so called constitutional obesity is not definitely understood. It is said that the maternal factor is more important in this connection, but how far it is due to constitutional cause or over-eating, remains to be settled.

The endogenous type of obesity is said to be more common in women than the exogenous variety. This is in conformity with the fact that sudden increase in weight is commonly observed in women at puberty, after child birth and at the menopause. In man this change during the middle years is less sudden. Nevertheless changes in the testes, hypertrophy

of the prostate and analogous changes in other endocrine glands may occur; this is probably due to a general lowering of the activity of the catabolic glands. As a result of this many people show a tendency towards accumulating fat.

Endocrine obesity.

The part played by endocrine glands in the causation of obesity is important and interesting. Obesity associated with thyroid deficiency with the clinical picture of myxœdema is well known. In these cases the classical features are coarse facies, thick lips, puffy eyelids, dry skin, etc. Thyroid deficiency is unquestionably responsible for a large number of cases. The pituitary gland also plays an important part in the causation of obesity. Hypopituitarism (Frolich's syndrome) is a commonly recognised condition. It has also been shown that so-called pituitary obesity can be experimentally produced by injury to the hypothalamic region. Langdon Brown (1931) stated that the pituitary plays an important role in the production of some forms of obesity, but the relationship of the pituitary gland to obesity is complicated by the fact that lesions of the hypothalamic region produce identical changes.

Gonadal insufficiency is also responsible for a certain number of cases. Furthermore there exists a close relationship between the thyroid, pituitary and the gonads. Lambie (1931) described various syndromes resulting from the interaction of gonadotropic, thyrotropic and growth-promoting factors of the anterior portion of the pituitary gland. The commonest example of these cases is a combination of pituitary and thyroid obesity. Such conditions as Dercum's disease (adiposis dolorosa) and the Lawrence-Biedl syndrome have been described.

In women, among the endocrine glands, thyroid and ovaries have been chiefly incriminated as the cause of obesity. There is no doubt that these glands play a great part in the life of a woman. Many cases of obesity in women are known to show a rapid loss of weight after thyroid treatment. The part played by the ovary is not definitely understood. In menopausal obesity ovarian hormone has given variable results.

The influence of insulin as a cause of obesity was originally suggested by Falta. Poulton (1931) discussed this theory of excessive production of insulin as a cause of obesity. It is suggested that diabetic patients treated with insulin develop obesity and that insulin along with carbohydrates helps to fatten lean people. The sugar tolerance is increased in some cases of obesity which is compatible with an increased output of insulin. This theory has not been universally supported.

The prognosis in obesity. Obesity is a disease which ordinarily shortens the span of life. The more marked it is, the more pronounced will be its effect upon the length of life. The statistics based upon the tremendous material at the disposal of the American life insurance companies prove that stout people die on an average of seven years earlier than those who are not obese.

Though we may laugh at the fashion of slenderness today, especially in the feminine sex, it must be welcomed as desirable. Of course, there are cases in which it is carried too far and becomes a menace to life and health. From a medical standpoint, it must be admitted that it may contribute to the longer life of the present generation.

2. Treatment of Obesity

As we are forced to admit that obesity is a condition which threatens life and shortens it, its development must be prevented and its existence must be done away with.

Prophylaxis and General.

Prophylaxis is first and most important in regard to obesity. It is far easier not to get fat than it is to lose excess fat. Unfortunately the layman is just as ignorant of normal weight as he is of the dangers of obesity. It is the physician's duty to enlighten him.

The only effective prophylaxis is to be familiar with normal weights, to record the weight frequently and to adjust the diet to fit the case. Nothing is worse than the ostrich-like behavior of some fat people. For fear of unwelcome discoveries, they prefer to go on growing fatter rather than to be weighed and learn the truth.

Naturally our first effort will be to reduce the food.

The Dietetic Therapy.

The caloric content of the diet is more important than its composition. The reduction in the fuel value of food, however, depends upon the degree of obesity.

The underlying principle is to give a diet of caloric value below that required for the optimum weight of the patient. As a general rule it should be 20 to 25 per cent below his normal requirement. Great care should at the same time be taken in preserving the proper nitrogen equilibrium of the patient which is of great importance. The diet should be arranged in such a way that the patient is forced to burn his own fat without the loss of his body proteins. This is not difficult to accomplish provided the diet contains sufficient proteins and is of a low caloric value. Usually, 1.5 gm. of proteins per kilo of the body weight will answer the purpose. It should also be remembered that carbohydrates in the food, besides being the chief source of energy for the maintenance of the body temperature and for the production of work, are most efficient protein sparers and every use should be made of them to preserve the nitrogen balance at an economic level. At least 50 per cent of the energy contained in the food should come from the carbohydrates. Thus, in calculating a reduction diet, the caloric value of the protein allowance should be calculated first and to this should be added an amount of carbohydrate sufficient to bring the caloric value up to the desired total caloric intake. Fats need not form an item of any importance in the dietary of obese persons.

According to Browning (1934) the essential factors in the choice of a dietary are:—

(1) *Preservation of nitrogen balance.* To do this the proportion of protein to carbohydrate should be about 1 gm of protein to 0.6 gm of carbohydrate with a total of not less than 60 gm of protein.

(2) *Minimum fat intake.* As already stated fat is cut down from the dietary so that the patient is forced to burn his body fat to supply the metabolic need.

(3) *Avoidance of hunger.* This can be avoided by giving high proportion of fruits and vegetables which have low caloric value.

(4) *Maintenance of water balance.* Sometimes the patient shows a tendency to store water in the tissue though he may be losing a good deal of fat. This is prevented by giving a salt-free diet and increased quantity of protein.

(5) *Adequate mineral and vitamin content.* The loss of mineral content with the use of the recommended diets is not great. If the fat is very much restricted, there is likelihood of the vitamins A and D being reduced below the standard level. This can be remedied by giving carotene and irradiated ergosterol. A cup of yeast extract once a day will supply vitamin B.

(6) *Physiological factor.* It is in many cases difficult to induce a patient to change to a diet of small quantity and simple character. Under these circumstances a slow reduction is preferable to a drastic one; the foods should be distributed over the various meals allowed, to make them as palatable as possible.

A very efficacious diet (modified by Douthwaite, 1934, from Williams) is given as follows:

On waking. A glass of water. *Breakfast.* Tea or coffee, sweetened with saccharine; one slice of dry toast, no butter; 3 oz of cold tongue, boiled sole, haddock, or whiting; fresh fruit. 1 *p.m.* A tumbler of water. *Lunch.* 4 oz. of chicken or meat (except pork), but no gravy, vegetables as given below, cooked without fat; salad without oil; one square of vita-weat; fresh fruit. 5 *p.m.* Tea without milk or sugar. *Dinner.* Bouillon; fish; 2 to 3 oz. of game or meat (except pork), no bread-sauce or bread crumbs; vegetables as mentioned below; salads; one slice of toast; dry wine; fresh fruits; coffee with

saccharine; condiments are allowed. Worcester and anchovy sauces, ketchup, pepper, mustard, vinegar, walnut pickle, horse radish, salt sparingly.

Vegetables allowed. Green vegetables (except peas), celery, seakale, asparagus, and salsify.

Another form of dietary has been devised by Evans and Strang (1929); one gramme of protein per kilo body weight is given along with some carbohydrate to maintain nitrogen equilibrium.

Evans and Strang Diet (*about* 650 *calories*); *Breakfast.* One egg and 1 oz. of bread. *Lunch.* One egg and 4 oz. of vegetables as below. *Dinner.* One cup of bouillon and 3 oz. of lean meat; 4 oz. of vegetables. Vegetables allowed: Lettuce, cucumber, spinach, asparagus, endive, celery, mushrooms, tomatoes, sprouts, watercress, cauliflower, radish, cabbage.

Water as much as needed; no fried foods; a teaspoonful of bicarbonate of soda daily.

It is essential to eat the whole amount mentioned in this diet. A modification of this diet is given by Dodds (1934) which allows only 8 calories per kilo. Though efficacious it is rather drastic. There is a tendency to acidosis with Evans and Strang diet, which may be relieved by the use of sodium bicarbonate.

Kenyon's modification (1933) is to give 1,000 calories which allows high protein, vitamin and mineral salt.

Breakfast. One portion of fruit: one egg and white of one egg; coffee, tea, bread substitute (for example, Heudebert's breadsticks). *Lunch.* 3 oz. lean meat, fish, or fowl; ½ pint 5 per cent. vegetables, one portion fruit. Tea. *Dinner.* 3 oz. meat, fish, or fowl and vegetables as at lunch. 9-30 p.m. Half-cup orange juice; 1 oz. bemax. In addition, 1½ glasses skimmed milk daily (for calcium content); one capsule haliverol t. i. d. (for vitamin content).

A fruit portion—one orange; half a grape-fruit; one medium apple; two medium peaches; one small pear; one cup strawberries; one cup blackberries.

It is important to control the body weight by weekly measurements. When the desired weight has been obtained, the diet is slowly increased.

Exercise.

The therapeutic factor next in importance to the restriction of food is the stimulation of the metabolism by physical exercise. Exercise hastens the metabolic rate and leads to increased oxidation of the ingested food, or, if sufficient food is not available, of the body fat. The exercise should be gradual and regular and should not be carried up to the point of exhaustion.

The other factor which also serves as a useful physical stimulus to metabolism is a cold shower or plunge bath and should be advocated where there are no contra-indications for it, such as, severe myocardial degeneration, arteriosclerosis and high blood pressure.

Endocrine therapy.

As it is possible to control obesity with dietetic restrictions in many cases, endocrine therapy should not be indiscriminately resorted to. Thyroid has been too frequently used to stimulate metabolism and reduce weight. In those cases where the basal metabolic rate is below normal, the patient will probably lose weight after thyroid medication. Unless properly controlled, even in cases of myxœdema, the weight after an initial fall may be increased after administration of thyroid. In thyrogenous obesity and other forms where metabolic stimulation is required, thyroid should be used in doses of half a grain twice daily; this is gradually increased to 1 gr three times a day. Some cases may require much larger doses. Other endocrine preparations such as pituitary, or combination of

thyroid and pituitary, or treatment by extracts of genital glands have been advocated, but the effect of such therapy after oral administration seems to be uncertain.

Drugs. It has been shown that certain nitrophenols are capable of causing a marked increase in both the temperature and oxygen consumption. Cutting and his co-workers (1933) found that by using a drug of this nature, which is 2: 4 dinitrophenol, the metabolic rate of animals could be raised by 50 per cent by a dose of 10 mgm per kilo body weight. In human beings the drug in doses of 3 to 5 mgm per kilo body weight daily raised the metabolic rate by 40 per cent and this was accompanied by an average loss of weight of 2 lb a week. As this compound has been known to be toxic, Dodds and Pope (1933) working with dinitro-o-cresol (dekrysil) found that it would produce the same effect in much smaller doses of 1 mgm per kilo daily. Douthwaite (1934) has used this drug in doses of 1 mgm per kilo thrice daily, and later once daily; though the constitutional effects were not marked with smaller doses, the loss of weight was very insignificant. These drugs are in the experimental stage and should, therefore, be used with caution.

In addition to these measures, other methods of therapy have a great bearing on the final success in treatment. Excretion of waste products of metabolism should always be favoured, and with this end in view the activities of bowels, kidneys, skin and lungs should be watched.

INFANTILE BILIARY CIRRHOSIS

Aetiology. Infantile biliary cirrhosis is a disease especially affecting young children in Indian cities. It is comparatively rarely encountered in rural areas and attacks Hindu children more often than Mohammedan. It is more common amongst the upper and middle classes than among the poorer classes. The disease generally starts during the first year of life, sometimes within a few days of birth, but seldom after the third year. The largest number of cases occur during the teething period, or during the mother's succeeding conception.

The aetiology of disease is unknown, but it is common in cities than in villages and is liable to run in families. Toxins in the maternal diet during pregnancy; dietetic errors in the mother and child; excess of fatty and farinaceous food: and mineral or vitamin deficiencies; have all been suggested as causal factors. In some children of a family, the disease has been averted by sending them to a better climate and surroundings, different from those in which the previous children contracted the disease. This may suggest that a climatic and environmental factor is involved.

Pathology. In the early stages of the disease, the liver is greatly enlarged. The enlargement is uniform and the surface smooth. Ascites is usually present, and the gall bladder is shrunken.

The characteristic histopathological change consists of formation of fibrous tissue, at first primarily within the lobules, but later extending to the sheaths and interlobular tracts. There is a characteristic multiplication of the bile-ducts which appears to be connected with the destruction of liver cells. In advanced cases there is great destruction of liver tissue, and in some areas, the whole field may appear to consist of fibrous tissue and scattered masses of granular debris.

In the early stages of the disease, jaundice is slight and is due to destruction of the liver parenchyma. The van den Bergh reaction is usually indirect, biphasic and delayed. Later the jaundice becomes more intense and is due to biliary obstruction, the serum giving a direct van den Bergh reaction.

Symptomatology. Early symptoms are vague and the disease is not generally suspected unless there is an indicative family history. Enlargement of the liver is noted at an early age and may attract the parent's attention. The liver is uniformly enlarged, not tender at first, but becomes slightly tender as jaundice develops. Usually the left lobe enlarges at first and may reach down to the spleen. The enlargement is progressive and the liver gradually fills the abdomen. Flatulence is marked, and the child becomes obviously ill and irritable, with loss of appetite, fever, and pronounced jaundice. The spleen is also commonly enlarged, but never to the same extent as the liver. Persistent pyrexia of an intermittent or low remittent type is usual.

In the early stages, the stools may lose their normal colour and as the disease

progresses they become white or clay coloured and often offensive. The urine is scanty and deeply bile-stained.

In the terminal stages there may be generalised anasarca, and the liver may contract. In some cases fatal hæmorrhages may occur from the bowels or stomach.

The disease usually runs a chronic course of from six to eighteen months. The prognosis in advanced stages is bad, and the majority of cases prove fatal inspite of treatment. In the earlier stages, the outlook is uncertain. Most cases in whom the liver is hard, tend to get worse and die. Generally speaking the harder the liver, the worse the prognosis.

Treatment.

In the early stages, a change of diet and climate gives perhaps the best chance of cure. Treatment is unsatisfactory and should be directed to relief of the early gastro-intestinal symptoms and to improvement of hygienic surroundings. The infant should be put on diluted or citrated milk, milk whey, or peptonized milk. Skimmed milk has also been advocated. Fruit juices should be given in amounts of 2 to 3 ounces daily. If after the gastro-intestinal symptoms have been relieved, ordinary milk does not suit the baby, artificial foods, such as Horlicks malted milk, Nestles milk or Mellins food may be added to skimmed milk in small amounts.

Medicinal treatment is of doubtful value. A prescription such as mercury with chalk 1/12 gr, salicin 1 gr, ipecacuanha 1/12 gr, extr. euonymus 1/6 gr, and sod. bicarbonate 2 gr, is usually given two or three times a day. Liver extract, emetine, hexamine, and an indigenous preparation, kalmeg have all been tried but with doubtful results.

GOUT

Gout is a luxury disease. Great stress has been laid on the inherited predisposition to gout and some go so far as to say that gout is always a hereditary disease and that all the other factors are merely determining agents, and in themselves incapable of giving rise to gout in the absence of hereditary taint.

Gout is an even more remarkable disease than has hitherto been recognised. Is it to be supposed that certain races have a special inherited predisposition to gout, while others are immune? Before such a view is accepted it must be clearly demonstrated that environment is incapable of accounting for the special frequency of gout in particular peoples.

Is it on account, of differences of inherited structure that the people of some parts of Scotland and of the North of Ireland (and even of India) are so free from the disease? And if so, how has this special racial immunity been maintained, in spite of the extent to which the inhabitants of the former two areas have a common ancestry with the people of England who suffer greatly from gout?

It will certainly be difficult to eliminate heredity as a factor in the causation of gout, unless it is clearly understood that the environment of the human being does not begin to act after birth, it has already produced a most important influence on the unborn babe. The child of a mother who has been living in the manner customary among gouty families has been nourished on what may be called gouty blood all through its intrauterine life.

That fact is of great importance in connection with the future constitution of the child. The alternative seems to be a frank recognition of the inheritance of acquired characters for it is hardly to be admitted that certain groups of people, in other respects exactly like each other, have been specially constructed in such a way as to make some of them susceptible to gout, and others insusceptible. On the other hand, wherever gout is common, there certain habits of life are common. These habits constitute the environment of the people and they influence even the unborn children.

Gout is not at all common in India, but the disease is of considerable importance to workers in India all the same. It is becoming more and more recognized in this country. A diagnosis of gout is often made in India when it is not at all justified; there are excellent examples of the diseases that are so often confused with gout.

Typical cases of gout are sometimes seen among the well-to-do classes in northern India and the writer has personal knowledge of such cases. They are hardly ever seen in the out-patient departments of hospitals.

LATAH

Latah is an unusual form of mental disturbance which is not infrequently

encountered amongst the native populations of the Malay Peninsula, the Indonesian and the neighbouring islands. It appears more often in women, especially at the menopause, and is rare before puberty. The condition persists for years but shows no tendency to get worse, and does not progress to insanity. It is not amenable to any form of therapy.

Symptomatology.

Victims of this disease, who have hitherto appeared quite normal, may suddenly in response to some stimulus, such as a loud sound or overt suggestion, pass into a peculiar hypnotic-like mental state, during which they may involuntarily perform certain movements or utter certain sounds or words. Patients in such a trance, which usually lasts only a few minutes, may be entirely at the mercy of their prompter, and may follow any lead given without regard to consequences. The trance usually leaves no ill after effects, though in some severe cases, the patient be left utterly exhausted, or may even faint. It is well known amongst the Malays that Latah victims never 'run amok', and are only very rarely involved in criminal acts. The disease does not progress and no effective treatment is known.

RUNNING AMOK

Running amok is a form of mental derangement, which when fully developed, causes its victims to exhibit fits of senseless fury, during which they may run mad and murder innocent people, often without any obvious reason whatsoever. The tendency to such derangement is greatest amongst the Malays, and the prevalence of the abuse of narcotic drugs such as *Cannabis Indica* are considered as aetiological factors. Chronic malaria has in recent years been strongly incriminated as an exciting factor.

The victim of this disorder usually nurses some grievance against a particular person and sullenly broods over it for some time. He then suddenly decides to destroy both that person and himself, and as many other people that come in his path as possible. Arming himself with a knife he sallies forth, stabbing any-body that he encounters, whether it be friend or supposed enemy.

Van Loon in Java reported that he found amok runners frequently suffering from infectious disease, malaria has also been incriminated. They exhibit symptoms of hallucinations and mental confusion, and are urged to run away and attack others in reaction to some imaginary danger and the apprehension and terror it causes.

Treatment consists of administering sedatives, and making a search for malaria, or other infective disease, which must then be adequately treated.

KORO

Koro, which is well known among the Chinese as *'Shook Jong'* is a condition which occurs among the Macassars and the Bunginese in the Celebes Islands. The affected patients, at regular intervals, get a strong and frightening sensation that the penis is being pulled up into the abdomen. The patient grasps his penis to prevent this happening, and gets others to help him. If not helped, the patient may die of an acute anxiety state. The penis may be tied to the leg with strings and pins, by the frantic patient in his efforts to avoid the supposed disaster, and this state may continue for days before it subsides. It is believed that local pathological conditions such as hernia, hydrocoele, elephantiasis of the scrotum, or œdema of the lower abdomen may be the causes which provoke the actual attack.

Treatment consists in administering sedatives and hypnotics, reassuring the patient, and dealing with any local pathological lesion that may be present.

PART II

REMEDIES USED AGAINST HELMINTHIC DISEASES

HELMINTHIC CONSIDERATIONS—SEROLOGICAL CONSIDERATIONS: IMMUNITY; DIAGNOSTIC TESTS—TREMATODES OR FLUKES OF MAN: GENERAL CONSIDERATIONS; TREATMENT WITH ANTIMONY COMPOUNDS; TREATMENT WITH EMETINE AND OTHER DRUGS; LIVER, INTESTINAL AND LUNG FLUKES—TAPE WORMS OF MAN: CLASSIFICATION; INTESTINAL TAPEWORMS; LARVAL TAPEWORMS—NEMATODES OF MAN: CLASSIFICATION; HOOKWORM; ASCARIS; TRICHURIS, STRONGYLOIDES AND ENTEROBIUS—TISSUE NEMATODES: FILARIAE DISTRIBUTION IN INDIA; LOA LOA; GUINEAWORM; ONCHOCERCA; TRICHINELLA—GENERAL CONSIDERATIONS OF ANTHELMINTIC DRUGS: DRUGS ACTING ON INTESTINAL HELMINTHS; DRUGS ACTING ON TISSUE HELMINTHS; MODES OF ADMINISTRATION; CRITERIA OF CURE; EXPERIMENTAL INVESTIGATION—DRUGS USED AGAINST HELMINTHS: MALE FERN AND ALLIED DRUGS; CARBON DERIVATIVES: TETRACHLORETHYLENE, CARBON TETRACHLORIDE, OTHER CARBON DERIVATIVES; THE ESSENTIAL OIL GROUP: OIL OF CHENOPODIUM, OTHER ESSENTIAL OILS; SANTONIN; THYMOL AND ALLIED COMPOUNDS: THYMOL, BETANAPHTHOL; RESORCINOL DERIVATIVES: HEXYL RESORCINOL, HEPTYL RESORCINOL; TRIPHENYL METHANE DERIVATIVES: GENTIAN VIOLET; MISCELLANEOUS ANTHELMINTICS—PRESENT POSITION OF ANTHELMINTIC DRUG THERAPY.

1. Helminthological Considerations

(1) Historical and General

General.

From time immemorial helminths were known to human beings, their effects on hosts, human and animal, were recognised and attempts were made to eradicate them by various drugs. In Egypt (1600 B.C.) a worm pathogenic in the human body was described. The Greeks (490 B. C.) were aware of infection by tapeworms and roundworms and laid down corresponding lines of treatment. Hippocrates diagnosed hydatid disease and suggested a technique for removal of the cysts. The Persian physician Avicenna (980-1036 A.D.) classified the helminths and listed many medicaments for expelling them, mentioning also the precautions in each particular form of treatment. In the middle ages, in Europe a distinct advance in the knowledge in anthelmintics was made. Nevertheless, it is only lately that the study of anthelmintic drugs has been taken up on scientific lines. The modern era of anthelmintic therapy is based on a comprehension of the biological and pathological processes of the parasites and a knowledge of toxicity and specific anthelmintic action of several important drugs. Moreover, the comparative study of toxicity and specificity of drugs seem to eliminate a few time-honoured so called specific drugs from the minds of physicians because of their high toxicity and lack of specificity. In their place have been found new drugs with lower toxicity and surer action and in many instances the need for a better chemotherapeutic agent has stimulated the synthesis of superior drugs.

The worms which have been recorded as parasites of man and domestic animals are numerous. They belong to a number of different divisions or phyla

of the animal kingdom, vary enormously in their habitat, habits, life cycles and modes of infection, and range from being practically non-pathogenic to being invariably fatal. Of the great number of species involved however, the great majority are so rare and unusual, as to be medical curiosities. Many others are of more or less local distribution, and only a comparatively few are normal and frequent parasites of practical importance. These belong to three principal groups falling under two quite distinct zoological phyla, the *Platyhelminthes* and the *Nemathelminthes*. To the former belong the flukes and tapeworms, and to the latter the nematodes.

Platyhelminthes.

The *Platyhelminthes* are usually flattened worms without any body cavity, the organs being imbedded in a sort of loose packing tissue. With a few exceptions they are hermaphroditic, *i.e.*, both male and female sex organs occur in the same individuals; the blood flukes, (schistosomes) constitute a striking exception. The *Platyhelminthes* are soft bodied and lack the resistant cuticle which is characteristic of the nematodes. The flukes have a well-developed branched digestive tract with only a single opening, the mouth. The tapeworms, on the other hand, have completely lost the digestive tract and live by absorbing, through the surface of the body, the semi-digested fluids in which they are constantly bathed in the intestines of the host.

Segmentation of tape worms.

The tapeworms which are parasitic in mammals have a further peculiarity in that as the parasite grows, the body undergoes segmentation, so that a chain of what are essentially complete individuals, except for organs of adhesion, is formed. The worm attaches itself by means of a head or scolex which is developed during the larval stage. Usually at a distance behind this organ of attachment, is the unsegmented portion known as the neck.

Shedding of segments.

After the worm has reached a certain length which varies between certain limits for each different species of worm, segments begin dropping off at the end as new ones are formed in the neck region, and this process continues as long as the worm survives. Anthelmintics frequently cause the worm to break off in the neck region, in which case a new chain is regenerated. In one group of mammalian tapeworms, the Diphyllobothriidae, the eggs are shed from the segments throughout a large portion of the length of the worm simultaneously; in *Hymenolepsis* the eggs are liberated from the terminal segments while still in the body of the host; whereas in the Taenias, the eggs are usually not shed at all inside the body of the host, except when a detached segment becomes broken or disintegrated.

Nemathelminthes.

Nemathelminthes. The nematodes are usually round in cross section, and possess a body cavity (not a true coelom) filled with a fluid. They are protected by a resistant and relatively impermeable cuticle of a chitinous nature, and are always bisexual, *i.e.*, with separate male and female worms. They possess a comparatively simple straight digestive tract with both oral and anal openings. Most of the intestinal nematodes produce characteristic eggs which pass out of the body with the faeces, but there are a few exceptions. *Strongyloides* produces eggs which hatch into embryos before being evacuated, and *Enterobius* (threadworm) usually does not shed its eggs at all, but instead, the females containing the eggs pass out of the body entire. For this reason enterobius infections cannot usually be diagnosed by ordinary methods of searching for eggs in the fæces, nor can the results of anthelmintic treatment be ascertained in this way. Only when a worm is accidentally crushed or disintegrated before escaping are the eggs found in the faeces. *Trichinella* produces embryos only, the eggs hatching out before leaving the body of the parent worm. These embryos bore through the mucous membranes of the intestine of the host, make their way through the tissues, and with the help of the blood stream lodge in the striated muscles in various parts of the body, where they encyst.

Distribution.

Many of common parasites of man and domestic animals have a practically cosmopolitan distribution, but others are limited by the distribution of suitable intermediate hosts, or of habits which make their acquisition possible. Thus most of the human infections and some animal infections are limited. The distribution of the species the larvae of which develop as free-living organisms, *e.g.*, hookworms and strongyloides is limited by climatic conditions of temperature and moisture. Many parasites are common to man and hogs, several to man and rats, and some to man, hog and rats.

In some cases helminthic infestations are seasonal, and very often so, as regards their acquisition. Thus liver flukes in cattle and sheep are, as a rule, acquired in early summer, and adults are found in the liver in the following winter and spring. Hook-worms, however, though only acquired during the wet season in countries like India, where there are several months of dry weather, are perennially present because the span of life of at least some of the individuals is several years.

Mode of transmission.

The mode of infection of helminths is very variable but in the majority of cases it is by way of the mouth, either with food or water contaminated with eggs of the parasites, or with the flesh of intermediate hosts in which the larval forms of the parasites have developed. A few parasites, especially the hookworms and their allies, strongyloides and schistosomes, enter by burrowing through the skin, and others by inoculation by the bites of intermediate hosts, *e.g.*, filariae. In most instances the eggs or larvae of the worms escape with the faeces, but the guineaworm exudes her embryos through a skin ulcer; the filariae are sucked up with blood by bloodsucking mosquito intermediate hosts, the eggs of lung flukes are coughed up, the eggs of some schistosomes escape with the urine, and the larvae of trichinella worms encyst in the musculature of the parent host and await being devoured by another host.

Damaging effects of helminths.

In a general way it may be said that a parasite damages its host in one or more of five ways:—

(1) By robbing it of food which has not yet been assimilated and utilised. This is of least importance, though sometimes the amount of food abstracted may be considerable, as in the case of large and numerous tapeworms. (2) By mechanical injury or obstruction of tissues, organs and ducts. The biting of the intestinal wall by hookworms, the burrowing in tissue by lung flukes, the injury to capillaries and irritation of tissues by the eggs of schistosomes, the perforation of the stomach wall by gnathostomes, the obstruction of bile ducts, by liver flukes, of lymph ducts by filariae, of the heart by *Dirofilaria* and even of the intestine by tapeworms or ascarids, and the pressure on nerve centres by *Coenurus cerebralis*. These are only a few examples. (3) By production of toxic excretions or secretions absorbed by the host. The effects of some are much more marked than that of others, *e.g.*, hookworms and *Diphyllobothrium*. Such worms may produce far more serious effects on the blood and nervous systems than on the digestive tract in which they live. (4) By providing portals of entry for infectious organisms and their toxins. The burrowing of ascaris larvae in the lungs, the bites of hookworm larvae in the intestinal wall, the perforation of the stomach wall by gnathostomes, etc., provide portals of entry for bacteria or other organisms and for the absorption of their toxins. (5) By interfering with the digestive processes. The round worms are known to produce an albumose which inhibits digestion of proteins. A similar mechanism may be present in case of hookworm damage.

Response to anthelmintic drugs.

Parasitic worms fall into two general groups as regards their reactions to anthelmintics. The nematodes and flukes respond to treatment with one group of drugs, which are classed here as the nematode group, whereas the tapeworms respond very feebly to these drugs, but are actively attacked by an entirely distinct group, which we therefore refer to as the cestode group. At first sight it might be expected that the flukes would show affinities with the tapeworms as far as their response to anthelmintics are concerned. Probably their affinity with the nematodes results from the presence of a digestive tract and the ingestion of blood, whereas tapeworms absorb only predigested foods through the body wall.

Although not strictly helminths, bots, which are the larvae of certain species of dipterous flies, need to be considered in connection with helminthic infections.

Cattle are subject to infection with two species of bots or warble flies, *Hypoderma lineata* and *H. bovis*. The adult bees, like flies deposit their eggs on the skin of the animals, whence they may be licked off by the cattle, or they may be left to hatch, the larvae penetrating the skin and causing a dermal rash. In any case the larvae eventually reach the oesophagus, bore through the mucosa, migrate through the body tissues, and eventually come to rest in the subcutaneous tissue of the back where they cause the well-known tumours.

(2) Prevention and Control

From the study of the life history of various helminthic parasites it is obvious that close association of man with animals is responsible for spread of a large number of helminthic infections among human beings. A certain number of helminths may occur in the adult stage of their life both in man and animals,

while others pass either the larval or adult stage in man, and the second part in animals. Thus we can classify helminths into three groups:—

1. Helminths living as adults both in man and animals.
2. Helminths passing larval stage in animals and adult stage in man.
3. Helminths occuring as adults in animals but as larvae in man.

Infection with helminths may only rarely be seen in man whereas it is quite common in animals. In these cases animals seem to act as 'Reservoir Host' passing infection to man from time to time. Going further into the life history of these parasites and studying the mode of infection of man it is realized that the control of helminthic infections in man is not possible without control of these among animals side by side.

Prevention of helminthic infections in animals is, however, a very difficult problem, particularly in India, where no care of sanitation or water supply of animals is taken. Animals eat un-cooked food, soil the food with their own dejecta and drink water from ponds and streams from which they infect themselves.

The general methods of prevention and control have been summarized by Leiper as follows:—

Control.

1. Medication by means of anthelmintics and therapeutic drugs.
2. Prevention of the entry of the infective stage by any of the following means:—
   - (*a*) Avoid exposure to infective soil. Use of boots recommended.
   - (*b*) Avoid open air bathing in places liable to contamination.
   - (*c*) Avoid use of unfiltered water for domestic purposes.
   - (*d*) Avoid use of uncooked foods.
   - (*e*) Avoid overstocking.
3. Destruction of intermediaries by chemical and other means.
4. Destruction of reservoir hosts.
5. Proper disposal of manure.
6. Clean stables.

These methods individually or collectively would greatly reduce chances of infections.

2. Serological Considerations

(1) Immunity

It has been shown during recent years that immune phenomena are elicited in animals as a result of infection with helminths just as with other groups of infecting agents. In infected animals it has been shown that humoral and cellular forces come into play to bear against the parasites as in other types of infection. These forces are manifested by the presence of certain serological changes, temporary or permanent, which are important like those in bacterial or other infections. Often the existence of a helminthic infection can be diagnosed by the demonstration of antibodies in the serum or by a skin test on conventional lines, the results obtained by these methods being characterised by their usual specificity. In some cases, the serum of an infected animal has been seen to protect another susceptible animal against infection.

Immunity against helminths may be natural or acquired. Natural immunity, exhibited

in cases where an animal resists a particular helminthic infection, may be present life long, or may be acquired with advance of age. Total immunity or absolute resistance against all parasites is unknown. It is observed that older animals of a susceptible species show resistance to infection with increase of age; nevertheless it is also seen that in many cases animals do not exhibit this immunity as they mature. In such cases it is often possible to demonstrate an immunity acquired by artificial infection or artificial immunisation with antigen prepared from the specific parasite. The host specificity found in animals where a particular parasite is found in a particular animal only, *e.g., Taenia saginata* and *T. solium* in man, may be an instance of natural immunity of other animals.

Natural immunity.

Acquired immunity.

It has been argued that the parasites which occur in the lumen of the intestine or biliary ducts, never passing through the tissues and thus having no chance to come into intimate contact with blood or tissue fluids, seldom give rise to any immunological phenomena; but there has been a conflict of opinion on this point. It is reasonable to suppose that parasites, which enjoy a circuit through the blood during their life cycle do call forth certain humoral changes; and parasites settling in tissues always stimulate certain changes in the tissues of the host, and consequent serological changes are responsible for the immunological reactions.

Relation of location of worm to immunity.

Chandler draws attention to a local immunity which is sometimes manifested. In his opinion this local immunity is different from locally manifested general immunity or part of the immunity in general. Parasites located in the skin or in the lumen or lining of intestines or biliary or other passages, are expected to give rise to this kind of local immunity, whereas those parasites imbedded in internal tissues or enclosed in body-cavities or lying in the circulation, are not likely to do so. He postulates that there are two phases of immunity in intestinal helminthic infections; (*a*) a reaction to parenteral migration or mucosal burrowing of the larvae before they grow to maturity and (*b*) a reaction to the adult parasites in the intestines. Parenteral migration such as occurs in hookworms, ascaris or strongyloides, stimulates a general immunity which is shared by the intestinal mucosa. Apart from this, another immunity may be developed against these migrating parenteral larvae due to localisation of the parasites. The local immunity is not responsible for the serological phenomena demonstrable in helminthic infections.

Local immunity.

Two phases of immunity.

These immunological reactions are of great diagnostic value in diseases such as hydatid infection, cysticercosis, trichinelliasis and some other helminthic infections which can hardly be diagnosed in latent stages without the help of these methods.

(2) Immunity in Diagnosis

As a rule the diagnosis of a helminthic infection is made from either the finding of the worms themselves, or their reproductive products, namely, eggs, embryos or larvae. Sometimes this direct method of diagnosis is not possible and in these cases the serological methods are resorted to.

As stated before, these serological and allied reactions depend on the development in the body of the host, of a specific immunity to an invading helminth. The diagnostic methods are therefore applicable to those infections where the helminth is intimately associated with the tissues and blood of the host. Thus hydatid disease, trichinelliasis and schistosomiasis, are readily diagnosed in a high percentage of cases. In other instances the reactions are not definite. In the case of ascaris infection the worm need not be an actual parasite at the moment of performing the test, since contact with this worm can sensitize the persons handling or examining them, the sensitization being caused by the emanations from the worms.

The antigens used for performing these tests are prepared in various ways. They may be simple saline extracts of the helminths or alcoholic extracts evaporated to dryness and subsequently dissolved in saline. In some cases the body fluids or extracts of particular tissues of the worm are used *e.g.,* of ascaris. In some other cases the metabolic products are used *e.g.,* hydatid fluid. Some workers have used hydatid scolices or their alcoholic extracts evaporated to dryness, and a solution is made in saline prior to use. Even extracts of snails containing cercariae have been successfully used in the diagnosis of schistosomiasis.

Preparation of antigens.

The four types of serological reactions adopted for diagnosis of helminthic infections are:—

(*a*) Complement-fixation test.

(*b*) Precipitin and flocculation test.

(*c*) Intradermal reaction.

(*d*) Precipitation reaction.

Complement-fixation tests.

Complement fixation test has been largely used in the diagnosis of hydatid disease, trichinelliasis, schistosomiasis, taeniasis, cysticercosis, etc.

In hydatid disease.

Fairley (1922) used the hydatid fluid with viable scolices obtained from sheep as an antigen in diagnosing hydatid disease. Dennis (1937) pointed out that Fairley's antigen was unstable and he prepared a stable antigen in a purified form from the hydatid fluid by precipitation of a protein with trichloracetic acid, purifying it once more and evaporating it to dryness. This powder is dissolved in physiological saline and passed through a filter to ensure sterility and further diluted before use. This antigen, he claims, is sensitive, specific, not anticomplementary and does not give false positive tests.

In trichinelliasis.

In trichinelliasis the test is often of value but intradermal and precipitin tests are found to be much more reliable. Complement-fixation test has been of special help in suspected cases of schistosomiasis in (*i*) unisexual infection; (*ii*) in chronic cases where owing to intense fibroblastic changes the eggs cannot pass out and hence a direct diagnosis is not possible; (*iii*) in the latter part of the incubation period before oviposition. In *Taenia saginata* infection the complement-fixation test is positive. In cysticercosis this test is positive in a number of cases no doubt but precipitin and intradermal reactions are much more reliable. In ascaris infection this test is of value where male worms are only present. In paragonimiasis it is useful in suspected cases of non-pulmonary infection where the worms are so deeply lodged that eggs cannot be evacuated in the excreta or by the skin lesion, to make a direct diagnosis possible.

In taenia saginata.

In ascaris and paragonimiasis.

Precipitin and Flocculation Tests.

The use of precipitin and flocculation tests lies in checking the intradermal test. Its value is stressed in cases of cysticercosis, trichinelliasis, hydatid disease apart from other infections. In the last case the precipitin reaction closely parallels the complement-fixation reaction.

Intradermal Tests.

Intradermal reaction is based on the fact that the skin acquires a sensitiveness to the body protein of the infecting helminth in some cases. The technique consists in injecting intradermally, an extract of the parasite tissue or fluid elaborated by the parasite or in placing the fluid or desiccated powdered tissue or a solution of it on skin which has previously been scarified. In sensitized persons there is an immediate local reaction in the form of an erythematous wheal, rapidly increasing in size and often developing pseudopodia, reaching its maximum in 15 to 20 minutes and beginning to fade in about 1 hour. There is also a delayed reaction, an erythema and induration around the site of injection or application of antigen; this delayed reaction is not of great importance according to the present consensus of opinion. In hydatid disease this test is of great value. Casoni's reaction is an intradermal reaction done with hydatid fluid and he concluded that the diagnosis should be based on the immediate reaction rather than on the delayed one. In trichinelliasis and filariasis this reaction is of much diagnostic value. Although under carefully controlled techniques false positives do not normally occur, it is always desirable to supplement the intradermal test with complement-fixation and precipitin tests.

Casoni's reaction.

In schistosomiasis.

Precipitation reaction is a non-specific test due to the excess of serum euglobulin elaborated in the animal body in the presence of certain disease producing organisms. Though this test has been utilised in India and China for the diagnosis of Kala-azar cases (Chopra's Antimony test and Napier's Aldehyde test), it has been applied for diagnosis of infection with *Schistosoma japonicum* and *S. mansoni.*

Summary.

It has been shown during recent years that in animals, infection with helminths, like infection with other infecting agents, is followed by the development of immune bodies. These immune bodies can be demonstrated by means of intradermal and various serological tests, namely, complement-fixation, precipitin, flocculation, etc. In some cases the serum of an infected animal can protect another susceptible animal against infection.

Immunity against a helminth may be acquired through natural or artificial infection or immunisation with antigens prepared from specific helminths, or it may be innate. An animal species may be resistant to a particular parasite, the immunity being present throughout life or may develop with advancing age.

It is difficult to say to what extent the immunity developing with age is innate and to what extent previous infections with the same helminth or with other antigenically allied helminth are responsible.

(3) Eosinophilia in helminthic infections

Eosinophilia is met with in infection with *Wuchereria bancrofti* and with *Loa loa* and at times with *Dracunculus* infections. It may also be present in other nematode infections especially *Ankylostoma duodenale* or *Trichinella spiralis*. It is believed by some to be an attempt on the part of the organism to neutralize toxic substances produced by the parasites. There is also evidence that eosinophilia may occur as a protective measure against foreign proteins and may be produced by injection of foreign proteins; a local eosinophilia at the site of injection results if injections are repeatedly given. Eosinophilia appears after a latent period which might suggest the possibility of its being an allergic response. The occurence of eosinophilia in anaphylactic attacks is well known and it occurs in asthmatic conditions of allergic origin.

3. Trematodes or Flukes of Man

(1) Classification

The important flukes of man are the following:—

I. Blood Flukes:—
   1. *Schistosoma haematobium.*
   2. *S. mansoni.*
   3. *S. japonicum.*

II. Liver Flukes:—
   1. *Clonorchis sinensis.*
   2. *Fasciola hepatica.*
   3. *Opisthorchis felineus.*

III. Intestinal Flukes:—
   1. *Fasciolopsis buski.*
   2. *Metagonimus yokogawai.*
   3. *Heterophyes heterophyes.*
   4. *Gastrodiscoides hominis.*

IV. Lung Flukes:—

Paragonimus westermani and other species.

Life cycles.

All the flukes have rather complicated life cycles. After being voided by the host, (in urine, faeces or sputum) the eggs hatch out into ciliated larvae which live a short aquatic free-living existence. These larvae soon penetrate the soft portion of the snails in which they undergo complicated development, finally resulting in cercariae. In the blood flukes, these cercariae are capable of penetrating the skin of man and normally begin their parasitic lives in that manner. In the other flukes of man the cercariae encyst either on vegetation (*Fasciolopsis, Fasciola*), in fresh-water fish (*Clonorchis, Heterophyes, Metagonimus*) or in fresh-water crustacea (*Paragonimus*), and thus gain ingress to man in his food.

Classification.

The following table giving the classification of Trematodes is after Clay G. Huff:—

Class Trematoda

Subclass Monogenea (parasites of fishes, amphibia, turtles)

Subclass Aspidogastrea (parasites of molluscs and other cold-blooded invertebrates)

Subclass Digenea

Order Prosostomata (hermaphroditic, mouth usually surrounded by an oral sucker; a ventral sucker may also be present; cercaria with unforked tail)

Suborder Distomata

Family Fasciolidae

**Fasciolopsis buski* (greater intestinal fluke of man, in Assam, China)

Fasciola hepatica (sheep liver fluke; occasionally in man)

Family Opisthorchiidae

**Clonorchis sinensis* (human liver fluke)

Opisthorchis felineus (common in dogs and cats of Europe; occurs in man in Prussia, Poland, Siberia, India, Japan, French Indo-China)

Family Dicrocoeliidae

Dicrocoelium dendriticum (a liver fluke of sheep; incidental in man)

Family Plagiorchiidae

Prosthogonimus macrorchis (in bursa Fabricii and oviduct of hens and ducks)

Ostiolum medioplexus (lungs of frogs; useful for trematode study)

Family Echinostomatidae

Echinostoma ilocanum (intestinal fluke of rats; rare in man)

Family Heterophyidae

Paryphostomum suprartyfer (in pigs and man)

**Heterophyes heterophyes* (intestinal fluke of man in Egypt, China, Japan, Korea, Formosa, and Philippine Islands; common in cats and dogs)

Metagonimus yokogawai (lesser intestinal fluke; also found in dogs, cats, pigs, birds)

Family Troglotrematidae

**Paragonimus westermani* (human lung fluke; also found in cat, dog, tiger, fox, mink, etc.)

Troglotrema salmincola (intestine of dog; associated with salmon poisoning)

Suborder Amphistomata

Family Gastrodiscidae

Gastrodiscoides hominis (common intestinal parasite of pig in Orient, sporadic in man, in Assam, Cochin, China)

Family Paramphistomidae

Watsonius watsoni (rare intestinal parasite of man; probably a parasite of monkeys)

Order Strigeatoidea

Suborder Schistosomata

**Schistosoma haematobium* (blood fluke which produces urinary schistosomiasis or bilharziasis in man)

**Schistosoma mansoni* (blood fluke which produces rectal or intestinal schistosomiasis in man)

**Schistosoma japonicum* (blood fluke which produces oriental schistosomiasis or katayama disease in man)

*Those occuring in man are marked with an asterisk.

The subject of treatment of parasites occuring in the tissues is of great importance and although considerable work has been done in this connection little progress has been made. The difficulties arise out of many factors. The absorption of the drug is essential for any action to be produced on blood and somatic worms, and since most of the anthelmintic remedies are toxic to the host when absorbed in more than very small quantities, the difficulty of finding a suitable drug to destroy the parasites is great. Helminthic parasites may

occur almost in every organ and tissue in the body, and some are more easily reached than others. The parasites lying in the liver are easy to reach since the drugs absorbed from the gut go through this organ first, and they reach it in high concentration. The parasites lying in the lungs can be reached by drugs given by inhalation, and parasites in the blood can be reached by drugs from the digestive tract or by injection. Parasites lying in the subcutaneous tissue, muscle, central nervous system, etc., must be reached indirectly by drugs circulating in the blood stream, which are therefore much diluted by the time they reach the parasites.

The principal human somatic or extra-intestinal helminths important from point of view of anthelmintic treatment are, (1) flukes, parasitic in the liver, bile ducts, or pancreatic ducts; (2) lung flukes; (3) schistosomes located in the mesenteric blood vessels; (4) tapeworm cysts in muscles, liver and other viscera and the central nervous system; (5) filariae situated in lymph glands or ducts. The embryos of trichinella worms during their wanderings in the blood stream and muscle tissue, before encystment in the muscle fibres may also be considered capable of anthelmintic treatment.

The drugs which are considered effective against these parasites are practically the same as those used against intestinal helminthic infections. During recent years a few more drugs have been added to the list, *e.g.*, antimony compounds, emetine, carbon tetrachloride, etc.

(2) Schistosoma or Blood Flukes

There are three species of Schistosoma parasitic in man. In the case of *S. mansoni* of Africa and tropical America and *S. japonicum* of China, Japan and Philippines, the eggs escape through the walls of the rectum, greatly irritating that organ and also the liver. In the case of *S. haematobium* of Africa the eggs escape by way of the urinary passages, causing irritation to these. After development in certain species of snails (usually of genus Bulinous) the larvae escape as bifid tailed cerceriae into stagnant water and usually infect man by actively burrowing through the healthy skin of people while bathing or washing. There are separate male and female parasites living together in the blood vessels, and since they are slender and small they are not frequently seen unless especially searched for *post-mortem*. Most of the damage to the host is brought about by the eggs. They stimulate the proliferation of adjacent host cells, particularly the epithelium. This results in papillomatous growths, which cause obstruction, necrosis, sepsis, ulceration, and fistulae.

Clinical Aspects: The onset is marked by dermatitis produced by entrance of cerceriae into the skin. It is followed by general symptoms, such as remittent or intermittent pyrexia, urticaria, abdominal pain, rigors, etc. There is pronounced leucocytosis with high eosinophilia at this stage. *Signs and symptoms.*

This stage is then followed by deposition of eggs which takes place in the bladder in the case of *S. haematobium* and in the rectum in the case of *S. mansoni* and *S. japonicum*. The symptoms in this state, therefore, depend on the seat of deposition of eggs which are responsible for severe local irritation.

In many cases there are no marked symptoms while in other cases suffering may be very great and may ultimately end in death. Early toxic symptoms, such as fever, urticaria, etc., may appear within a month of infection; in other cases symptoms may appear after from 3 months to two years or more. The most striking symptom of deposition of ova in the mucous membrane of bladder is passage of blood at the end of micturition generally in the last few drops; clots may be present; there is frequency of micturition, and polyuria aggravated by exercise. A dull sense of suprapubic oppression is present and burning pain accompanies the act of micturition. Urinary calculi may be formed around the eggs which serve as nuclei. Sepsis of the bladder may occur and consequent cystitis may cause considerable suffering. In the terminal stages, such complications as dilatation of ureters, hydronephrosis, papillomata which may become malignant, occur. The eggs may sometimes be deposited in such situations as kidneys, prostate, seminal vesicles, rectum, vagina, lungs and brain, producing symptoms pertaining to the organ involved. Urinary *Complications.*

fistulae opening in the perineum and posterior surface of scrotum are met with. The patient becomes anaemic, wasted and debilitated. Ultimately untreated cases succumb due to one of the complications or superimposed infection.

Diagnosis.

Diagnosis depends on finding ova with a terminal spine, in the urine. The presence of haematuria in endemic areas is suggestive. Cystoscopic examination may help.

Prognosis in mild infections is good. In severe chronic cases it is bad, but treatment with antimony has greatly improved prognosis.

Intestinal schistosomiasis.—The localizing symptoms are diarrhœa, dysentery (Metazoal dysentery), tenesmus sometimes choleric diarrhœa. The course in infections with *S. japonicum* is usually graver and more rapid than with *S. mansoni*. Splenomegaly and hepatolineal fibrosis may occur in a few cases. In others cirrhosis may result. In the terminal stages large growths in the passage causing obstruction, prolapse of papillomatous growths or fistulae in the anal region may result. Death may ultimately result from exhaustion, cholæmia or superadded infection.

Visceral schistosomiasis.—Infection with *S. mansoni* is frequently followed by splenomegaly. This is common in Egypt and in some localities 20 per cent of infants have enlargement of spleen. The condition resembles Banti's disease and is associated with anaemia, fever and wasting.

The liver in early stages is enlarged and haematemesis is often present. The patient often dies of hepatic cirrhosis but death is usually caused by pulmonary complications. The lungs may be involved even though there may be no clinical symptoms. Prognosis is good in mild infections, but with marked hepatic enlargement and ascites, it is bad. Diagnosis is made by finding characteristic ova in the fæces.

Treatment.

On account of their location these parasites can only be reached *via* the blood stream, and are usually attacked by means of intravenous injection of anthelmintic drugs. Very striking results have been obtained from the use of tartar emetic, which seems to have a specific effect on both the worms and the eggs. Some less toxic preparations of antimony have been recently tried with beneficial results, such as 'Fouadin', Anthiomaline etc.

(3) Treatment with Antimony Compounds

Schistosomiasis has been successfully treated by injections of tartar emetic. It was at one time thought that there was no means of destroying these parasites but Christopherson (1918) first tried, and showed that injections of the double salts of antimony are successful in eradicating both kinds of African schistosome infection—*S. haematobium* and *S. mansoni*. The efficacy of antimony injections against the related parasite *S. japonicum* has also been shown. The routine followed by Christopherson is to begin with 1/4 grain (0.015 gm) doses for children and 1/2 grain (0.03 gm) for adults, dissolved in 20 min. of distilled water and then diluted with an equal quantity of normal saline. The dose is increased by half a grain up to 2½ grains (0.1 gm) unless a reaction is produced before that time. Injections are given every other day, the dosage being kept between 2 to 2½ grains (0.13 to 0.16 gm) until 25 to 30 grains (1.7 to 2.0 gm) have been given. This amount is usually sufficient to cure an adult; children are given proportionately smaller doses, the maximum dose being on the basis of 0.003 gm (3 mgm) per kilo body weight. In out-patient clinics in Egypt over 100,000 patients were treated annually, the treatment used being a course of 12 injections, given three times a week, and totalling 22.5 grains (1.5 gm) of tartar emetic; this cures 80 to 97 per cent of cases. The effects of the treatment are most striking. After a few injections the vesical pain and the scalding sensation disappear. Blood is also absent from the urine though sometimes it may persist a little longer. After the full course the urine becomes clear and normal in colour; this change probably corresponds with the cessation of the activities of the parasites and the cure of the disease. This course is suitable for the

Tartar emetic.

Dosage.

early stage of the disease when the patient is in a good state of health. In old-standing cases, where they are weak and emaciated, a less intensive course is desirable. A slight coughing at the end of the injection is not a contra-indication for continuing the treatment, but if nausea, vomiting, abdominal pains, giddiness and diarrhœa supervene the patient should be given a few days rest. If more than 25 grains are required it is better to give a second course after an interval of a fortnight or more. Lampe (1926) gave to out-patients 150 to 250 ccm of a 1.0 per cent solution in 6 to 7 weeks; to in-patients, a more intensive course of 200 to 240 ccm in 4 to 5 weeks was given. As a rule no untoward symptoms occurred. Lampe's conclusions are in accord with those of Christopherson and he considers 25 to 30 grains (1.6 to 2 gm) spread over 6 to 7 weeks the best treatment. It is best to continue treatment for a week or 10 days after all ova and leucocytes have disappeared from the urine after centrifugalising.

Usually after the injections have been given for a week or 10 days, improvement begins. In *S. haematobium* the vesical pains disappear, the urine clears up, and so does the scalding sensation when the urine is passed. In the case of *S. mansoni* and *S. japonicum* the blood in the fæces decreases, fever disappears gradually and the asthenia and malaise improve. In favourable cases eggs may disappear in 10-15 days. In severe cases dead ova are passed intermittently for weeks and months after the parent worms are dead. Degeneration of miracidia is seen early, generally after about 12 grains have been given. The ova become shrunken, shrivelled and blackish and later do not hatch out in water. When this stage is attained the parent worm is killed and the ova are sterilised *in situ*. As a rule 30 grains (2.0 gm) of the tartrate administered in from 28-30 days form the curative dose. The rapidity and permanence of cure are less dependent on the total amount of antimony administered than on a regular tri-weekly series of injections. Re-examinations of patients who have been given a course of 12 injections show that 80 per cent to 97 per cent are cured completely.

Criterion of Cure.

The criterion of permanent cure seems to be not yet standardised. The general customary methods *i.e.*, the microscopical examination for the eggs has not proved sufficient for all cases. Some authors adopt eosinophilia as an indication for infection or cure where no other worm affection has been observed. The Fairley test remains positive too long for it to serve as a control while on the other hand the method of testing by hatching of miracidia if any, seem to be an improvement.

Mode of action.

The drug kills not only the adult worms but penetrates the shells and kills the ova deposited in the tissues. This can be demonstrated by adding tartar emetic to 6 ccm of warm water at 133°F. to which a little urine containing ova has been added. It is found that if the antimony salt is not added, the ova begin to hatch out in 4 to 5 minutes and that most of them have hatched out in an hour. If, however, a grain of tartar emetic is added to the water only half the ova hatch out and a good many are found to be dead, half in and half out of the shell. If the concentration is further increased very few embryos hatch out and even the few found swimming do not survive long. Under the microscope one can watch the darting, pulsating, corkscrew movements of the miracidia slowing down in contact with tartar emetic solution. Microscopic examination of eggs voided during injections of antimony show that they have undergone degeneration. It is a curious fact that pentavalent antimony derivatives have no effect in bilharziasis. Hamilton fairley (1924) showed that the lethal effect of tartar emetic *in vitro* was greatly enhanced in the presence of human serum. Everything points to the fact that tartar emetic is a powerful therapeutic agent in the trea-

ment of all schistosome infections. Even in patients undergoing surgical treatment a course of antimony is desirable.

Relapses.

Hamilton Fairley (1924) was of opinion that tartar emetic, in a proportion of cases merely reduced the parasitic level and converted cases of frank clinical schistosomiasis into passive carriers in whom ova are shed in small quantity and at such irregular intervals as to escape detection on one or more isolated examinations. He tried the drug in *S. spindalis* in goats and got results which suggested that the antimonyl tartrates were less effective than emetine. These results, however, cannot be applied to the human disease and investigations in Egypt show that emetine is not as effective as tartar emetic. Day (1924) showed that comparatively small doses of antimony (25 grains of tartar emetic) are required to kill the ova present in the tissues, and that reappearance of ova in the urine after small doses, is due to the fact that a sufficient quantity of the antimony salt has been given to kill the ova but not the adult parasites, which have only been temporarily affected by these small doses. Relapses are due to lack of judgment with regard to dosage; if a sufficient quantity of antimony compounds are given no relapses occur. It has been shown that 20 per cent of cases relapse after 2 years with 0.7 gm, 15 per cent have viable ova after 1.3 gm; after a full course of 2 gm 3.5 per cent still remain infected. The usual cure rate is 79 per cent. In uncomplicated cases, as a rule, 25 grains (1.6 gm) produce a complete cure, but the total quantity of the drug varies largely in different individuals and every case should be treated on its own merits. Even in the worst cases the mortality is reduced to half.

Disadvantages.

With the extension of the tartar emetic treatment, considerable defects were revealed which make mass treatment difficult. Not all patients can tolerate the long treatment on account of the toxic effects of this drug. Irregular treatment even with a large dose makes the result uncertain. Besides one should always bear in mind the technical dificulties, such as the trouble of preparing the solution before use in freshly distilled water on each occasion. Further on storage the toxicity of the solution may increase. The impossibility of injecting it intramuscularly necessitates emetine treatment in the case of small children and patients with bad veins. To overcome these difficulties sodium antimony tartrate was at first tried and later Fouadin was evolved.

Fouadin (Neo-antimosan).

Fouadin. Clinical trials have shown that preparations in which antimony is present in a trivalent form, as in tartar emetic, were effective in schistosomiasis. A compound named antimosan, which could be administered intramuscularly, had a remarkable action in schistosomiasis. This drug is well tolerated intramuscularly though it was not entirely painless; it could also be given by the intravenous route and was less toxic than tartar emetic. It is superior to stibenyl and stibosan. These observations led to the preparation of fouadin (neoantimosan).

Purified oxide of antimony is used in its preparation and it contains 8.5 mgm of trivalent antimony per ccm. It is isotonic with the tissues and is neutral in reaction; the sodium salt is used instead of the potassium salt in its preparation. Animal tests show that in therapeutic doses it is not toxic. In 80 patients of which 68 were infected with *Schistosoma haematobium,* 7 with *Schistosoma mansoni* and 4 with mixed infection of both, complete cure was obtained in all except 5 cases. Total dosage required was from 40 to 50 ccm of fouadin per 60 kilo body weight, given in 9 to 10 intramuscular injections. The fact that the action of antimony continues even after the completion of the treatment could be seen from the progressive degeneration of the eggs. In 3.5 per cent of all the cases, headache and nausea, and in 5 per cent, vomiting, in the second half of the treatment were observed.

The absorption of fouadin solution administered intramuscularly is completed within 10 minutes. Fifty per cent of antimony administered is eliminated through the urine and only approximately 4 per cent through the faeces. *Absorption.*

Khalil (1931) used fouadin in the treatment of Schistosomiasis on a mass scale in the following manner: injections were chiefly given intramuscularly, 100 ccm ampoules being used and a special apparatus for rapidly filling the syringe was employed. The patients were asked to rest for 2 hours after the injection. *Dosage.* The doses in an adult of 60 kilo were: first day 1.5 ccm, 2nd day 3.5 ccm, 3rd day 5 ccm and then 5 ccm every other day until a total of 40 ccm was administered. If living ova were still present, the treatment was continued but more than 11 injections were rarely necessary. The treatment lasted 2 weeks as compared with 4 weeks with tartar emetic treatment. In mass treatment practice, fouadin therapy was usually extended to 19 days and that with tartar emetic to 29 days. In favourable cases the treatment was shortened by giving 4 or even 5 injections on successive days. Vomiting occurred in 0.36 per cent and abscess formation was seen only in two instances among approximately 20,000 injections. Bradycardia was present in some cases but far less than after antimosan; the pulse was not reduced by more than 12 beats per minute. On the whole it could be said that fouadin is tolerated better than tartar emetic and its effect on the eggs is identical.

Intramuscular injections of fouadin are practically painless; the only disadvantage is that fouadin is a more costly drug than tartar emetic.

Cases of vesical schistosomiasis suffering simultaneously from cutaneous leishmaniasis require a great number of fouadin injections to influence the latter complication in addition to the former, 13 to 24 intravenous injections being given. The high dosage is well tolerated and the urine is freed from ova at the conclusion of the treatment. Intravenous therapy does not have any advantage over the intramuscular route, either as regards the duration of treatment or the total quantity of the drug necessary.

The standard treatment evolved in Egypt has proved successful. Adults *Standard course.* are given 9 to 10 injections. The first two injections, given on successive days being of 1.5 ccm and 3 ccm respectively. The subsequent injections are 5 ccm each and given every other day. Khalil has attempted to shorten the duration of treatment; commencing with a dose of 3.5 ccm followed by doses of 5 ccm, the first 3 or in suitable cases 5 injections being given on successive days, and the remainder every other day till a total of 8 to 11 injections or more are given.

The drug is contra-indicated in hepatic cirrhosis, acute pyelitis, cardiac disease, and pregnancy. *S. haematobium* infestations respond more easily than those of *S. mansoni.*

In comparing the efficacy of tartar emetic with fouadin the quantity of antimony used should be borne in mind. According to the dosage scheme of Khalil the following figures are of interest:—

	No, of injections	Time	Total dose	Mgm of Sb
Fouadin	9	19 days	43.5 ccm (1 ccm= 8.5 mgm Sb)	370
Tartar emetic	12	28 days	1.35 gm (36.5% Sb)	493

This shows that in tartar emetic therapy more antimony is used.

Anthiomaline.

Other Antimony preparations. In recent years the new preparation *anthiomaline* has been used in North America and remarkable results are reported. This preparation is better tolerated than tartar emetic and is said to be effective in schistosomiasis. It is supplied in 2 ccm ampoules of a 6 per cent solution containing 0.02 gm of antimony. The initial dose is 1.5 ccm and the maximum dose 4 ccm; a total of 65 ccm is given in an adult. In children of twelve years the initial dose is 0.5 ccm and the maximum dose 2 ccm. It is best given by the intravenous route on alternate days.

Stibophen.

Stibophen is a 6.3 per cent solution of sodium antimony bis-catechol-3:5-disulphonate, which can be used by the intravenous and intramuscular routes. The initial dose in adults is 1.5 ccm, 3.5 ccm on the second day, 5 ccm being the maximum dose; a total of 40 to 75 ccm is given in an adult. For children, the initial dose is 0.5 ccm and 3.5 ccm is the maximum dose and a total of 47.5 ccm is given. An average course lasts 29 days.

Prophylaxis.

Bathing in and drinking of water of rivers, ponds and canals should be prohibited. People should be warned against wading through, or fishing in infected localities. Swamps, when slightly brackish, are safe. Drinking water should be boiled. The diffusion of disease should be prevented by prohibiting soiling of water with excreta, especially of infected individuals. The intermediary mollusc and the free cercariae should be attacked by periodic drying of irrigation channels, etc., and the use of chemical manures. Such cercariacides as sulphate of soda tablets should be used for treating drinking water; for bathing water lysol, creolin, or cresol (1 in 10,000), may be used. Chlorine, ordinarily used for sterilizing water (1 in a million), has no lethal effect upon living cercariæ. Lime (1 in 1,000) is effective in exterminating the intermediary host and kills cercariæ. Application of steaming jet and copper sulphate (1 in 20,000) are toxic to snails. Eggs remain viable in faeces for ten days. Mass treatment will help to eliminate carriers; educational propaganda is important. It should be remembered that the disease occurs in dogs and other domestic animals, and snail vectors live in inaccessible situations. In heavily infected snails, the genital glands are infected and they do not propagate.

Intermediary molluscan hosts: *Bulinus contortus, B. dybowski* (Egypt and North Africa), *B. innesi* (Sudan), *B. truncatus* (Palestine), *B. forskali* (Mauritius and Kenya), *B. globosus* (Sierra Leone, Nyasaland), *Physopsis africanus* (Natal), *B. tropicus* (S. Africa), *Isodora ovoidea* (Zanzibar), *Physopsis nasita* (Kenya) *Planorbis dufourii* (Portugal and Morocco). In the genus *Bulinus* the body of the snail contains red haemoglobin. The shell is spiral, not operculated, and the opening sinistral. (Manson Bahr).

(4) Treatment with Emetine and other drugs

Although emetine hydrochloride does not kill the bilharzial cercariæ (*S. spindalis*) *in vitro* in 1 in 100 dilutions, in the presence of serum it kills them in dilutions of 1 in 160,000. The lethal effects of both emetine and tartar emetic are greatly enhanced in the presence of human serum.

Emetine hydrochloride.

Intravenous and intramuscular injections of emetine have been used in the treatment of *Schistosoma haematobium* by many authorities, and have succeeded in permanently eradicating the infection. Six daily injections of 1 grain (0.06 gm.) each are given, followed by an intermission of six days, when a second series of 6 injections is given. In children, doses of ½ gr (0.032 gm) are often sufficient. No living parasites or ova can be found after the fifteenth injection. Emetine is specially indicated in patients who are intolerant to antimony. The drug acts on the ova which show degenerative changes; it also kills the adult worms gradually. Diamantis (1918; 1921) reported a number of cases of *S. haematobium* infection which were cured by emetine injections. He gave the drug intravenously in doses of 0. 12 gm, 10 to 12 injections at intervals of 3 to 5 days; 0. 8 to 1.05 gm of emetine in all, produced a cure. It is also effective against *S. mansoni.* In

urinary schistosomiasis the length of treatment and dosage are both important. Harkness (1920) gave a total of 14 grains (1.0 gm) and a further total of 20 gr. without effecting a cure. Cawston (1922) recommended emetine dissolved in 20 minims of a 1 per cent solution of carbolic acid, intramuscularly for 8 consecutive days and then thrice weekly for three weeks. Cardiac depression occurs in the second and third week but it can be avoided by giving digitalis. He gives emetine in preference to antimonyl tartrates in children and young people in whom it is difficult to get into veins. According to him curative doses of emetine for schistosomiasis is double that given for amœbic dysentery. *S. japonicum* has been cured with emetine injections starting with ½ grain and increasing the dose to 2 grains daily, as toleration developed; two courses of 15 grains each with an interval of one week are recommended. Cawston (1926) held that unless very large doses of emetine are given uninterruptedly emetine cannot be depended on in schistosomiasis. Such doses are very risky and are not to be recommended.

Emetine periodide.

Emetine periodide has been recommended in the treatment of *S. hæmatobium* in doses of 1 grain (0.06 gm) three times a day. The drug does not produce any toxic symptoms and appears to have curative properties. Auremetine given intravenously is also said to be effective. Some workers suggested the use of antimony along with that of emetine but experience shows very little advantage in such a mode of treatment. Khalil suggests that as emetine is a depressant drug, its use in Schistosomiasis should be limited to those cases where amœbic dysentery is simultaneously present.

Emetine with antimony.

It will be seen from the above review of the literature that emetine should only be used in those cases of schistosomiasis which are intolerant to antimonyl tartrates or in children in whom it is difficult to find the veins. In cases of intestinal schistosomiasis complicated by amœbic dysentery, emetine is indicated. Its dosage should be carefully regulated and if toxic symptoms appear it should not be continued.

Emetine in other helminthic infestations. Emetine has been found useful in infestations of the liver with *Fasciola hepatica* and *F. gigantica.* Kikilko and Imamura (1920) found daily injections of emetine useful in clonorchis infections.

Emetine injections have been recommended in the treatment of dracontiasis. Tournier (1923) treated 17 cases of this infection by giving emetine intravenously and by the mouth, with good results. This has not, however, been confirmed by other observers. Emetine in daily injections is said to be valuable in ameliorating symptoms produced in paragonimiasis, but these cases need careful watching for the onset of toxic symptoms. Potassium iodide and tartar emetic injections are preferable.

Emetine preparations have been tried against filarial infections, especially of *W. bancrofti,* but without effect.

(5) Liver, Intestinal and Lung Flukes

Liver flukes.

Clonorchis sinensis lives in the bile ducts of the liver. It is a flat and partly transparent parasite, about half an inch in length, and several times as long as broad. The eggs enter the intestine with the bile and escape with the faeces. After development in snails, the larvae encyst in certain species of fresh-water fish, and infection results from eating uncooked infected fish. The infection is common in man and fish-eating animals in countries where fish is habitually eaten raw. Infection is met with in Indo-China, Japan and China. It produces thickening of bile ducts, enlargement of spleen, and cirrhosis of the liver. Rarely it may be found in the pancreas, duodenum and stomach. In mild infections, night blindness and epigastric distress occur, while in severe cases chronic diarrhoea, recurring

jaundice, anasarca, and cachexia are met with, ending in death after several years. There is leucocytosis up to 30,000, eosinophilia upto 40 per cent. Eggs may be found in the duodenal juice removed by a duodenal tube.

Treatment is unsatisfactory but intravenous injections of fouadin (1.5 to 5 ccm (10 or more) are useful; Gentian violet has also been tried. In daily doses of 3 grains (0.10 gm), in divided doses an hour before meals, it is quite effective. In children dosage is calculated on basis of 0.01 gm per year of age. Non-surgical drainage of bile (continuous) by duodenal tube gets rid of the eggs and toxic matter and gives temporary relief.

Fasciola hepatica is the cause of liver rot in sheep; it occurs in man in Central Europe, Syria, England, South America and China. The parasite is found in the portal vein and in subcutaneous abscesses; it may produce epileptiform convulsions. Infection occurs by eating water plants on which the cerceriae encyst, from infected streams. There is no effective treatment but emetine injections and neostibosan may be beneficial.

Opisthorchis felineus occurs in Persia, Siberia, Philippines, etc., in man, dog, cat and pig. In man, it produces the same symptoms as *Clonorchis* but it is not markedly pathogenic.

Intestinal Flukes.

Fasciolopsis buski is a large flat worm, an inch or more in length and about half as wide. It occurs in man, pigs, dogs, and other animals in India, Assam, China and Malaya. Its embryos develop in snails, and after development, the larvae leave the snail and encyst on water vegetation, such as water caltrop, infection resulting from eating such vegetation uncooked. The parasite is found in the small intestine, rarely in the stomach, and produces alternate diarrhoea and constipation with offensive stools, œdema of face, legs and abdominal wall. Symptoms may resemble that of peptic ulcer. B-naphthol, carbon tetrachloride, tetrachlorethylene and hexylresorcinol in usual doses, are effective.

Heterophyes heterophyes is a very small intestinal fluke, hardly larger than a pin head. It reaches the human host by encysting in the flesh of certain species of fish, which is often eaten raw. This infection is also common only in the Far East. Enormous numbers of these parasites stick to the mucous membrane of the small intestines giving rise to diarrhoea; symptoms of cardiac beri-beri occur on account of eggs occuring in myocardium. Effective treatment is by thymol or by oleoresin of male fern.

Metagonimus yokogawai occurs commonly in Balkan States and in the far East, Korea and Japan. The parasite is found in upper part of small intestines of man, dog and cat. The metacercaria encyst under scales of fish which is eaten raw in Japan and may give rise to diarrhœa. Treatment with thymol and aspidium is effective.

Gastrodiscoides hominis, normally a pig parasite, occurs in man in India, Assam and Indo-China. Its life history is not definitely known but is probably similar to that of *Fasciolopsis buski.* Treatment with tetrachlorethylene is effective.

Lung Flukes.

Paragonimus westermani is met with in the Far East, India and U.S.A. It occurs in man, wild canidae and mink. This fluke is rather egg-shaped, almost as thick as wide, reddish brown in colour and about half an inch in length. After development in certain species of snails the larvae encyst in fresh-water crabs, and infection results from eating uncooked or undercooked crabs, a practice which is common in certain parts of Japan where the infection is prevalent. Young flukes escape through the host's intestines into the abdominal cavity, penetrate the diaphragm and then enter the lungs. Other organs may also be the seat of development. Small brown spots occur in lungs and scattered burrows are visible. Eggs are coughed up in sputum in enormous numbers and it is often associated with tuberculosis of lungs; eggs also occur in fæces and rarely in spinal cord where they produce transverse myelitis. Hundreds of these flukes have been found in psoas abscesses. Symptoms start with distress in chest, cough, and brown rusty sputum containing thick operculated eggs; irregular haemoptysis may occur. Dull pain in the abdomen, diarrhœa, enlargement of liver, clubbed fingers, epileptic symptoms, hemiplegia, aphasia, visual disturbance, and cutaneous ulceration may occur. This condition can be easily diagnosed by finding eggs in sputum and faeces, and leucocytosis.

Treatment is unsatisfactory but seven day courses of injections of emetine 1 gr each intramuscularly may do good. Intravenous injections of 0.5 to 1.0 ccm of a 4 per cent solution of emetine hydrochloride once or twice daily and intramuscular injections of 5 ccm of a 2.5 per cent solution of prontosil at the same intervals until the ova show degenerative changes or disappear; this takes usually 7 to 17 days. The symptoms improve. Emetine, however, is a toxic drug and should be used with caution. Lipiodol injections into the bronchi have been tried. The sputum should be burnt and sale of crab forbidden to prevent infestation.

4. Tapeworms of Man

Tapeworms infect man both as adults and larvae. Many species have been reported as attacking man. However, there are only four adult tapeworms which may be considered normal human parasites, namely, *Diphyllobothrium latum* the fish tapeworm; *Hymenolepis nana*, the dwarf tapeworm; *Taenia saginata*, the beef tapeworm; and *Taenia solium*, the pork tapeworm.

(1) Classification

The following table giving classification of Cestodes is after Clay G. Huff: (A Manual of Medical Parasitology)

Class Cestoidea, Platyhelminthes
- Subclass Cestodaria (in fishes)
- Subclass Cestoda
- Order Pseudophyllidea

Family Diphyllobothriidae
- **Diphyllobothrium latum* (broad fish tapeworm of man)
- *Diphyllobothrium erinacei* ('Mansoni', occasional larval infections in man)
- *Diphyllobothrium cordatum* (accidental parasite of man)
- *Diphyllobothrium mansonoides* (probably adult of *Sparganum proliferum* of man)
- *Diplogonoporus grandis* (rare parasite in Japan)
- Order Tetraphyllidea (in fish, amphibia, reptiles)
- Order Cyclophyllidea

Family Taeniidae
- **Taenia solium* (pork tapeworm of man)
- **Taenia saginata* (beef tapeworm of man)
- *Taenia pisiformis* (cats and dogs)
- *Taenia ovis* (dogs)
- *Taenia hydatigena* (dogs)
- *Taenia taeniaeformis* (cats)
- **Echinococcus granulosus* (hydatid in sheep, cattle, man)
- *Multiceps multiceps* (gid worm; rare in man)
- *Multiceps serialis* (dogs)
- *Multiceps gaigeri* (dogs)

Family Davaineidae
- *Raillietina tetragona* and other spp. (chickens)
- *Raillietina asiatica*, *Raillietina celebensis*, *Raillietina madagascariensis* } rare parasites of man.

Family Anoplocephalidae
- *Bertiella studeri* (monkeys, chimpanzees, man)
- *Moniezia expansa* (sheep, cattle)

Family Hymenolepididae
- **Hymenolepis nana* (dwarf tapeworm of man)
- *Hymenolepis diminuta* (rats, mice, man)

Family Dilepididae
- *Dipylidium caninum* (dogs, cats, man)

*Asterisks indicate that larval infection are common in man.

(2) Intestinal Tapeworms of Man

Diphyllobothycum Latum. The fish tapeworm is a very large, broad, flat worm reaching a length of 15 to 30 feet or more. It has a slender head and neck, the former with two longitudinal sucking grooves instead of the usual four rounded suckers. The segments are *Diphyllobothrium latum.*

broader than long, and have a rosette-shaped uterus, containing the brown eggs near the centre. This worm produces eggs, usually in large numbers, which resemble the eggs of flukes in that they are provided with an inconspicuous operculum at one end, but the shell is much thinner. The intermediate hosts are, successively, water fleas (Cyclops) and fish. Infection can occur only when undercooked infected fish is eaten.

Generally the digestive symptoms caused by this worm are not many and may not be even noticed, Abdominal pain, loss of weight or other symptoms are the same as occuring in other *tænia infections*.

This parasite, however, has been associated with pernicious-type of anæmia, of a severe degree. Although the infection is very common in many countries, anæmia develops only in a small percentage of the cases. The anæmic condition improves after liver therapy even without the removal of the worms. There has been much discussion with regard to the role of *diphyllobothrium* in the production of this anæmia during recent years, but our knowledge on this point is still not definite.

Hymenolepsis nana.

Hymenolepis nana is a very minute tapeworm, parasitic in rats and mice as well as in man. It is seldom as much as an inch in length, and very slender. When expelled in the fæces it resembles a little strand of mucus. It is undoubtedly the commonest human tapeworm, and is often present in large numbers. Unlike other human tapeworm, it undergoes complete development from egg to adult without change of host. The larval stages are developed in the intestinal villi, as the eggs are shed from the segments while still inside the body of the host. Diagnosis can be made by the usual fæcal examination. If the eggs are discovered in the fæces and the six little hooks which are characteristic of most tapeworm eggs are seen, it is very commonly incorrectly diagnosed as a *Taenia* infection, and the physician is at a loss to know why he is unable to expel any part of such a worm by the anthelmintics which are always efficient in removing at least the greater part of *Taenia*.

The parasite in heavy infections may produce nervous and digestive symptoms especially in children who suffer from pain, diarrhoea, epileptic attacks and insomnia.

Taenia saginata.

Taenia saginata is in most countries the commonest large tapeworm of man. It reaches a length of from 10 to 15 feet or more, the ripe detached segments are shaped like pumpkin seeds and are about an inch in length; the head is provided with four suckers but is devoid of hooklets, and is about the size of a medium-sized pin head. The detached segments pass out with the fæces, only occasionally rupturing before leaving the body, so that one cannot depend on diagnosing the infection by searching for eggs in the fæces. The worm develops into the larval bladderworm stage in cattle, and human infection results from eating infected beef which has not been thoroughly cooked and still retains its red colour.

Taenia solium.

Taenia solium is similar to *T. saginata* but is smaller and has a head which is armed with a crown of hooklets as well as the four suckers. The ripe segments which are passed in the fæces can be distinguished with certainty from those of *T. saginata* by the number of lateral branches of the uterus, which occupies most of the segment. In *T. saginata* there are 13 or more of these branches on each side, while in *T. solium* there are only 12 or less. The intermediate host of *T. solium* is the pig and infection comes from eating raw or underdone pork. The pork tapeworm is a more dangerous parasite than the beef tapeworm because the larval bladderworm stage is capable of development in man as well as in pigs, and this may happen when a ripe segment is regurgitated into the stomach. If this worm is found it is advisable to give treatment at once.

The presence of the parasites in the intestines causes no distinct lesion in the early stages. Diarrhœa and hunger pains not infrequently develop and loss of weight may occur. Appendicular colic has been reported due to clogging of the appendix with shed segments (proglottides). The parasites possibly produce some toxic substance which may be the cause of digestive disturbances and neurosis seen in a few cases.

Difficulties in dislodging of worm.

All tapeworm infections are difficult to cure because the heads of the worms are deeply imbedded in the mucous membrane and are often not reached by the anthelmintics used. The main bodies of the worms are paralysed or killed and the movements of the intestine tend to drag them away, commonly breaking them off just behind the head, which then soon reproduces the whole worm again. It is for this reason that careful preparation of the patient by means of a liquid

diet and purges is necessary in order to increase the chances of dislodging the head.

The preliminary preparation of the patient in case of tapeworm infection must be thorough. The routine of treatment is described under aspidium. The patient should keep in bed after taking the drug. The stools should be passed in a chamber pot half full of water so that the worm can be recognised; this should be examined carefully to see if the head has been passed. In case the head is passed no more anthelmintic should be given but the stools should be watched to make sure that there is not a multiple infestation. If only segments have been passed the treatment should be repeated in full after 10 to 14 days. If there is doubt about the expulsion of the head, it is best to wait for 8 to 10 weeks, watching the stool in the meanwhile for reappearance of segments. If no ova or segments can be detected the head has been expelled. If *T. solium* is found, prompt treatment should be given since there is danger of cysticercus infection.

Preliminary Preparation.

The cestode group of anthelmintics includes the phloroglucinol series, *i.e.*, male fern which is entirely successful in dwarf tapeworms, and also kousso and kamala; pomegranate bark or its active principle pelletierine which should be employed when male fern fails; the active principles of areca nut (arecoline); peps or pumpkin seeds are remedies against *T. saginata.* Other less important agents are cocoanut, butylchloral hydrate, oil of turpentine, brayera, etc. Multiple doses of caprocol crystoids are effective. (See Hexylresorcinol).

In recent years carbon tetrachloride, tetrachlorethylene, hexylresorcinol, gentian violet (medicinal), sostol (an acridine compound) have all been used and seem to be almost as effective as the time honoured remedy filix-mas. These drugs are preferred now-a-days as the unstable nature of the active principles of male fern tends to make the results uncertain and attended with toxic manifestations.

Carbon Tetrachloride and other drugs.

(3) Larval Tapeworms in Man

Larval tapeworms (bladderworms, coenuri or hydatids) in domestic animals may be of importance from two points of view, namely the health of the animals themselves, and the danger of transmission to man.

Cattle are subject to infection with the cysticerci of *Taenia saginata,* known technically as *Cysticercus bovis,* producing a condition popularly spoken of as measles. The bladder-worms, about half an inch in length, are imbedded in muscles. When under done meat containing these parasites is eaten by man, *Taenia saginata* infection results.

Cysticercus bovis.

Hogs are parasitised by the cysticerci of *Taenia solium* technically called *Cysticercus cellulose.* These resemble the bladderworms of cattle, but are frequently present in enormous numbers. These bladderworms will develop in many other animals also, including man, but the hog is the most frequent host, and the most important from the standpoint of human infection.

Cysticercus Cellulose.

Somatic taeniasis occurs in India. The embryos migrate to any organ especially muscles of limbs, tongue, neck or ribs, lungs, liver, heart, eyes and brain, and form cysts. These may be present in large numbers and are discovered accidentally, there being no ascertainable history of tapeworm infection. In the brain, the cysticercus becomes enclosed in a wall of neuroglial tissue, small round cells and a few plasma cells being present beyond this and between the normal brain tissues. The presence of the cysts in these tissues may produce serious disturbances as result of pressure. Cysticercus has been described as one of the causes of epilepsy among British soldiers in India. The fits are of Jacksonian type and symptoms may resemble those of a cerebral tumour. Psychical states and loss of memory may occur after a variable period determined by resistance of the host; the parasites die and often become calcified (in 3 years or longer).

Diagnosis is made by palpable cysts in tissues; excision of one will show characteristic central milk spot. When cysticerci are calcified, X-ray examination may be useful.

In the case of both these species, prevention consists in keeping cattle and hogs away from access to human fæces containing segments of the adult worms. Human cysticercosis is an accidental complication, auto-infection being caused by ingestion of eggs, or possibly by regurgitation of segments of *T. solium* into the stomach. (Manson-Bahr).

Treatment.—The anthelmintic treatment of these infections is not hopeful at present. Male fern, kousso, kamala, and pomegranate bark have all been tried but are useless. Intravenous injections of antimony tartrate have been tried without much effect. The embryos show a special predeliction for the brain, and localisation and removal of single cysts has been tried. Luminal and bromides may be helpful in controlling fits.

Cases of cysticercosis have been treated with fantorin (G.L.), the scale of dosage that has been worked out is as follows:—Initial dose may be 1 ccm to 1½ ccm increased by 1/2 to 1 ccm up to 5 ccm given intramuscularly. A total of 40 to 50 ccm of fantorin may be administered. Clinical evidence of improvement may be apparent at the end of this course.

Echinococcus granulosus.

Echinococcus granulosus is the normal parasite of dog, wolf and other carnivores. The intermediate stage is normally passed in sheep, goat and cattle. Man becomes accidentally infected only with the larval stage of the parasite by ingesting infected food or water or as a result of close association with dogs, whose skin may be harbouring the eggs.

The embryo is liberated in the intestines, and penetrates the intestinal wall and may reach the liver (60 per cent cases) lungs or other organs of the body, such as kidneys, spleen, heart etc.

The symptoms depend on the organ involved, which suffers grave injury and destruction as a result of pressure. Liver and lungs are commonly involved. Serious conditions may result from rupture or infection of the cysts.

The diagnosis, if cysts are near the surface, is made by the typical thrill present; if punctured with a needle scolices and hooks are recognized but this is a very unsafe procedure, as anaphylactic reactions may occur. The complement fixation test is reliable. The intradermal test is positive in 90 per cent of cases. A few drops of the prepared hydatid fluid from a sheep are injected intradermally; a reaction appears in 10 minutes in form of a large wheal surrounded by erythema. The test remains positive years after the surgical removal of the cyst.

Treatment.—On the same lines as cysticercosis.

5. Nematodes of Man

Of the nematodes, there are a large number of species which have been recorded in man, but the only ones which are sufficiently common to warrant consideration here, are the hookworms, (*Ankylostoma duodenale* and *Necator americanus*); *Ascaris lumbricoides; Trichuris trichiura; Strongyloides stercoralis; Enterobius vermicularis; Trichinella spiralis; Wucheria* (several species); and the guinea worm, (*Dracunculus medinensis*).

(1) Classification

The following table giving classification of Nematodes is after Clay G. Huff from 'A Manual of Medical Parasitology':—

PHYLUM NEMATHELMINTHES
Class Nematoda
Subclass Phasmidia
Order Rhabditida
SUBORDER RHABDITINA
Rhabditis, Turbatrix (rare parasites of man)
SUBORDER STRONGYLINA
Strongyloides stercoralis
Heterodera radicicola (plant parasite; eggs found in human feces)
Ternidens deminutus (common in man in parts of Africa)
Oesophagostomum (common in monkeys, rare in man)
**Ankylostoma duodenale,* hookworm
**Necator americanus,* hookworm
Ankylostoma braziliense, hookworm
Trichostrongylus colubriformis (rare in man; common in some ruminants)
**Enterobius vermicularis,* pinworm
SUBORDER ASCARIDINA
**Ascaris lumbricoides,* roundworm of man
Order Spirurida
SUBORDER SPIRURINA
Gnathostoma spinigerum
Gongylonema pulchrum (accidental parasite of man)
**Wuchereria bancrofti,* Bancroft's filaria
Wuchereria malayi
**Onchocerca volvulus,* cause of onchocercosis in man
Dipetalonema perstans
Mansonella ozzardi
**Loa loa,* eye worm
**Dracunculus medinensis,* guinea worm
Subclass Aphasmidia
Order Chromadorida
Order Enoplida
**Trichinella spiralis,* trichina worm
**Trichuris trichiura,* whipworm
Dioctophyme renale (rare in man, frequent in dogs, wolves, horses, cattle etc.)
Class Nematomorpha, "hariworms", rare in man
Class Acanthocephala, "thorny-headed worms", rare in man.
*Asterisks indicate that worms are common in man.

(2) Ankylostomiasis

Uncinariasis, Hookworm Disease

Hookworms.

The hookworms are widely distributed in all moist tropical and sub-tropical countries, and are the commonest human helminthic parasites. They are common in Southern Europe, less so in northern countries. Anaemia in Cornish mines in England is due to this infection. They are very prevalent in Egypt, South China, Malaya and India.

They inhabit the small intestines especially the jejunum, less so the duodenum and rarely the ileum, where they attach themselves by powerful buccal armature to the mucous membrane and injure the intestinal wall. They thus obtain nourishment and suck blood producing anaemia and other symptoms.

Morphology.

There are two species, *Ankylostoma duodenale* and *Necator americanus* both similar in size and general appearance, habits, and life cycle. They are about 1/3 to 2/3 inch in length, slender and reddish in colour when living, but dirty white when dead, as after being expelled by anthelmintics. The female produces a never ending stream of eggs which pass out in the fæces. In the body of the host, the embryos in the egg do not develop much, but on leaving it and under suitable conditions, the embryos hatch out in two days. This organism feeds voraciously on organic matter, and in two weeks it moults twice. The larvae then become torpid and in this condition are easily killed. Man is infected by these infective larvae boring through the healthy skin of the foot, legs or other parts of the body coming in contact with them. After entering the body, they are carried by the blood stream to the heart and then to the lungs; they then bore into the alveoli. From here they make their way up the bronchial tubes and trachea to the throat, are swallowed, and reach the small intestines and attain maturity in about six weeks after infection. *Ankylostoma duodenale,* has a more northern distribution than *Necator americanus.* The former is the prevailing species in Europe, North Africa and North China, while the latter is the prevailing and often almost exclusive species in North and South America, Africa south of the Sahara, Southern Asia and Australia.

Mode of infection.

Symptomatology.

The site of penetration of the skin by hookworm larvae may become the seat of dermatitis, known as 'ground itch,' especially in the case of *Necator americanus.*

There may be hundreds of the worms in the intestines without producing any symptoms whatsoever. Grave symptoms are not common, but may occur in some cases. In the tropics in patients suffering from dyspepsia, debility and anaemia, the physician should think of hookworm infection and if detected, treatment should be given at once. If infection is allowed to persist, severe anaemia may result. The essential symptoms of ankylostomiasis are progressive anaemia, which if unchecked, may produce fatty degeneration of the heart and death from collapse. Oedema may be localised or general.

Some patients complain of persistent epigastric pain and appetite may be defective and taste may be perverted ; diarrhœa (with imperfectly digested food) may occur, or there may be constipation. The stools are reddish-brown but seldom contain pure blood. Temperature may be constantly subnormal or there may be irregular or intermittent fever. Later pallor of skin, puffiness of face, lassitude, shortness of breath, vertigo, dimness of vision, mental depression and apathy, haemic murmurs, and retinal haemorrhages may occur.

Subacute infections may occur especially in Europeans.

Diagnosis.

Diagnosis is easily made by finding ova in the stools. Floatation method and concentration technique may be used and test made for occult blood. The grade of infection runs from 1,000 or more worms.

These symptoms develop insidiously. The anæmia is of the secondary type, hypochromic and microcytic in character.

Anaemia in hookworm infection.

There are different views regarding the manner in which anæmia is produced in this condition:—(1) Chronic loss of blood through feeding of the parasites and hæmorrhage from the spots from which the worms release their hold. (2) Hæmolytic toxin produced by the worms. (3) Unfavourable dietetic influences by interfering with the digestion of proteins. (4) Bacterial infection through the portals opened up by the bites of the worms.

Ankylostoma duodenale causes a more severe degree of anæmia in the host and is less susceptible to anthelmintic treatment than *Necator americanus.*

Distribution of infection in India.

Infestation with necators is common in Southern India; in northern parts of India, ankylostoma is common; infections are generally of mixed types. Milder infestations such as those containing 100 to 500 eggs per gramme of fæces are harmless. Experienced workers say that about 100 worms are required to produce any pathogenic effects and 500 to 1,000 worms must be present for at least six months to produce well marked symptoms; 3,000 to 4,000 may be present when severe degrees of anæmia exist. The other school holds that a small number of worms such as 10 or 20 may affect general health and working power.

They therefore advise wholesale treatment. Infections are present both in tropical and subtropical zones all over the world. In India the heaviest infestations are found in places which experience heavy rainfall, *e.g.*, Assam, Burma and Malabar. Moderate infestations are encountered in Bengal, Behar. the United Provinces and the Southern Bombay coast. Eighty to 100 per cent of infections occur in the country and 91 per cent in the urban areas with an average only of 10 worms.

Treatment.—Tetrachlorethylene, oil of chenopodium, carbon tetrachloride and thymol, are drugs which enjoy the reputation of being almost specific in the treatment of hookworm infestation (see description of these drugs). Recently hexylresorcinol in 1 gm doses has been found to be a very promising drug. Tetrachlorethylene and hexylresorcinol have become the drugs of choice. After an interval of 10 days after the treatment, the stools should be re-examined, and if ova are found, the treatment is repeated. During convalescence after severe infection, the diet should be gradually increased, so that the digestive functions get re-established, otherwise diarrhoea may occur. To combat anæmia large doses of iron should be given, *e.g.*, ferrous sulphate in doses of 12 to 18 grs daily for three weeks or longer. Blood transfusion 200 to 300 ccm, is useful in severe cases. The diet should be balanced and liberal in all its constituents.

Creeping eruption.—A linear eruption caused by the larvae of certain cat and dog hookworms. The larvae penetrate the skin and eruption progresses at one end larvae being seen just ahead of the lesion. The eruption is frequently seen on legs of children. Treatment is by freezing the advancing lesion with ethyl chloride.

Prophylaxis.—Faecal contamination of soil and water should be prevented by having proper latrine arrangements and disposal of night-soil. Larvae want air and earth for development, and if fresh stools are not mixed with earth they die. Infective stages of ankylostomes are killed by direct sun-light. When soil is infected the feet should be protected with some covering to prevent infection. If ankylostome dermatitis is present, some soothing ointment or antiseptic foot bath may be used. Application of strong salicylic acid solution in collodion, or methyl alcohol or 3 per cent and salicylic acid in ethyl alcohol are useful.

(3) Ascariasis

Ascaris lumbricoides is a very common human parasite, inhabiting the jejunum. The worms lie motionless and curled up in bundles so that the gut is stuffed up with them. Toxic symptoms produced are due to *ascaron*, a mixture of albumoses and peptones. (What is probably the same species is a very common pig parasite). Its eggs pass out of the body and an embryo develops within the eggs in the course of a week or two. A female ascaris may contain about 27 million eggs and after fecundation, about 200,000 are discharged daily. The eggs on soil become embryonated in four to six weeks time. Infection results from swallowing such eggs. *Ascaris lumbricoides.*

In China and Japan, where night-soil is used for cultivating gardens, the eggs are ingested with uncooked vegetables; in India, polluted earth or drinking water is the usual vehicle. When swallowed, the eggs hatch out and the embryos burrow into the mucous membranes, make their way to the lungs, and thence reach the intestines in the same way as do larval hookworms. The adults are eight to twelve inches or more in length, and as large around as a lead pencil. Sometimes no males are present, in which case unfertilized eggs are passed, different in appearance from the fertile eggs.

The larvae, in passing from the capillaries, incite a local allergic reaction. This allergic exudate may cause consolidation in the lungs and thus cause ascaris pneumonitis.

The passage of the worm gives rise to intolerable itching at the anus, and vomiting of the worms may produce œdema of glottis. There may be little or no symptoms; on the other hand, in children, it may produce interference with nutrition, capricious appetite, restlessness, foul breath and mental irritability, vague abdominal pain, nausea, and severe urticaria.

As a result of migratory habits, the adult worms may penetrate into any accessible passage and cause serious local symptoms, *e.g.,* into the appendix, bile ducts, gall bladder, pancreatic duct, nasal sinuses, middle ear, and larynx. In the intestines, the adults cause little local injury. When numerous they tend to aggregate in clumps and may cause obstruction. Nutritional disturbances may be produced as a result of inhibition of the digestion of proteins by pepsin and trypsin. A substance of the order of primary albumose named *'Ascarase',* found in the tissues of the worms, combines with these ferments and inhibits their action on proteins. Infection with ascaris is very prevalent in many insanitary tropical countries and is often associated with ankylostomes.

Diagnosis is made by microscopic examination of the stool, if necessary, by the egg concentration method. Eosinophilia is often present. Cutaneous reaction to ascaris antigens are of little use but the 'scratch test' with powdered ascaris is more reliable but it is often negative. X-rays are useful.

Treatment.

Santonin and oil of chenopodium are usually effective. The day before the treatment a saline purgative such as sodium phosphate or sulphate, or magnesium sulphate may be given. The following morning the anthelmintic is given in divided doses. Santonin usually combined with calomel is given at night, followed by a saline the following morning. The worms may not be expelled till 2 or 3 days after treatment. Both these drugs produce toxic effects.

In recent years hexylresorcinol (crystals in gelatine capsules) has been found to be the drug of choice because of its efficiency, safety and convenience of administration. The dose is 1 gm for an adult and for children 0.1 gm for each year up to ten years. The drug is administered on an empty stomach, and food is withheld for four hours. It is, however, a costly drug and hence not suitable for mass use, in comparison with a combination of santonin and chenopodium, the results in the latter cases being almost equally satisfactory. It is advisable to repeat the treatment after a week or two to make sure that all the worms have been expelled.

Recently phenothiazine has been recommended in 8.0 gm doses as an effective drug in the treatment of ascariasis (see description later on).

Prophylaxis.

Prophylaxis consists in good sanitation. Vegetables, grown in places where night soil is used as manure, should not be eaten raw. Eimeran, a coal-tar derivative containing sulphur is said to be effective in killing ascaris eggs, and may be used in latrines as an antiseptic.

(4) Trichuris, Strongyloides and Enterobius

Trichuris trichiura.

Trichuris trichiura is a whip-shaped worm, the head end being drawn out into a fine filament, comprising over one-half the length of the worm. Its total length is about two inches. It inhabits principally the cæcum and adjoining parts of the large intestine as well as the appendix, and affixes itself very firmly by threading its head into the mucous membranes. Its position far back in the intestinal tract and its firm fixation account for its resistance to anthelmintics. Embryos develop inside the eggs after they are passed, and infection results from swallowing such ripe eggs. Polluted water and vegetables act as vehicles for the eggs, but flies also carry them. After entering the body the eggs hatch and direct development occurs without migration to the lungs. Trichuris infections are as a rule incurable.

The infection is generally symptomless but in heavy infection the symptoms may be epigastric pain, vomiting, constipation or mucus diarrhœa, fever, flatulence, anorexia and loss of weight.

Treatment.

Many drugs have been used in the treatment of *Trichuris trichiura* or whipworm infections but none except one has been found to be satisfactory. Santonin and calomel 2 to 3 gr of each, oleo-resin of aspidium, thymol in doses of 15 gr, tetrachlorethylene, carbon tetrachloride, oil chenopodium with a saline purgative afterwards, have been found only partially efficacious; hexylresorcinol in doses of 1 gm without any after purge does not seem to be any superior. The only known drug which is efficacious against whipworm is *leche de higueron,* or hugerolatex, the sap of *Ficus laurifolia* and *F. doliaria* in South America. The dose for an adult is 2 oz (60 ccm) taken preferably on an empty stomach, followed by a glass of water. It is most successful if a saline purge is taken on the previous evening and again two or three hours after the administration of the anthelmintic.

Strongyloides stercoralis.

Strongyloides stercoralis is a very minute parasite, little over 2 mm long, and extremely slender, inhabiting the duodenum. Its eggs hatch before leaving the body of the host, so that only embryos are ordinarily found in the fæces. The embryos either develop directly into long slender infective larvae or into free-living adults, both males and females; the latter in turn produce the infective larvae. The larvae enter the body and reach the intestines in the same way as do the hookworm larvae. Only the females occur as parasites the males are very rarely found. These adult females embed themselves in the mucous membrances and thereby usually escape the action of anthelmintics. The infection is widely distributed in tropical countries especially in wet regions, but is seldom as common as hookworm or ascaris. It frequently causes a persistent diarrhoea. Moderate secondary anæmia may sometime be produced. *Strongyloides* often occurs in association with hookworms or independently; this infection is commoner in the Punjab and Rajputana. The females burrow into the intestinal wall and are difficult to dislodge.

Specific action of gentian violet.

While many drugs have been tried, gentian violet (medicinal) only has proved to be an effective strongyloidicide. The dye is given before meals in enteric coated tablets 1 gr thrice daily up to a total of 50 grs. For refractory cases, 2 ccm of a 1 per cent solution is put into the duodenum by a tube. For pulmonary infection and for severe late stage intestinal cases, intravenous administration of 0.5 per cent aqueous solution in doses not exceeding 25 ccm on alternate days is resorted to, for a period not exceeding 10 days. For such therapy the patient should be in a hospital and under careful supervision.

Enterobius vermicularis.

Enterobius vermicularis, the threadworm or pinworm, inhabits the colon, cæcum and lower portion of the small intestine. It is probably the commonest human parasite. The females are about one-half inch in length, slender, white, and sharply pointed at both ends. The males are much smaller and are usually overlooked. The adult female when full of eggs, migrate out of the anus, causing severe itching. The finger nails of children frequently crush the worms and become filled with eggs as the result of scratching. When the eggs reach the mouth *via* the fingers or in any other way, the worms hatch, pass to their habitat in the intestine, and grow to maturity. Enterobius infection may cause no disturbance except pruritus ani, which may lead to eczema as a result of constant scratching. Abdominal pain and convulsion in children may sometimes be due to these worms. Migration of the worms up the vagina and uterus may set up salpingitis, etc. Appendicitis may in rare cases be due to these worms.

Treatment.

The situation of these worms in the intestine renders them to some extent immune to orally administered anthelmintics, and usually enemata are necessary to entirely eliminate them, together with precautions to avoid reinfections. Since the adult female worms escape when filled with fertile ripe eggs, the infection tends to die out soon in the absence of reinfection. It is essential with any course of treatment to prevent reinfection. This is best accomplished by tying a cloth well smeared with zinc oxide ointment or unguentum hydrargyri, over the anus and between the buttocks. This should be replaced morning and

night, and worn for three weeks. This method insures against self reinfection. Ordinary cleanly habits are necessary to prevent reinfection from others. The treatment, therefore, resolves itself into two parts: (*i*) Use of suitable anthelmintics orally or in the form of enemata. (*ii*) Prevention of reinfection by the use of soothing and antiseptic ointments in the anal region at night and by paying strict attention to personal hygiene.

Tetrachlor ethylene.

Of the drugs used orally, salol, thymol and few others are worth mentioning though they have been replaced in recent years by drugs like tetrachlorethylene, hexylresorcinol and gentian violet. Tetrachlorethylene has been used in doses of 4 ccm for adults and over 64 per cent of cures have been claimed after a single treatment.

Gentian violet.

Persistent treatment is necessary to kill the young worms as they mature. The eggs probably do not live for more than two days under ordinary environments and prolonged treatment is necessary to prevent reinfection. Gentian violet is the drug of choice, 1/2 grain (0.0325 gm) being taken three times a day about one hour before meals for fourteen days, discontinued for seven days, and the course repeated again; or one grain may be taken three times a day for seven days, rest for seven days, and then the course is repeated. The results must be checked by seven consecutive examinations of anal swabs. All infected persons in a family should be simultaneously treated because reinfection often occurs in families. Rigid prophylactic measures should be taken by all infected members during the treatment period *i.e.*, clothes worn should be boiled and anal region should be thoroughly cleaned. Hands and nails should be scrubbed every morning before handling food. Toilet seats should be washed daily. The infections persist tenaciously in families.

Hexylresorcinol.

Hexylresorcinol has been used in hard gelatin capsules orally, supplemented by enemata of an 1-1,000 solution. For an adult the dose is 1 gm orally followed by a saline purge after 2 hours; 8 to 16 oz of the solution is given per rectum and is retained for about 30 minutes. Two or three combined courses of treatment at weekly or fortnightly intervals are necessary to produce a cure. Enemata of ether and liquid petroleum jelly (ether 20.0, vaseline liquid 100.0), have also been used in treating cases of threadworms. Two or three enemata daily are used for a week and a second course given after an interval of a week.

Enemata.

The enemata frquently used are of various kinds and are used at first daily and later on bi-weekly or weekly. The patient should be put in knee-elbow position and large enemata of 1 to 2 quarts of tepid or warm fluid are slowly given. The solutions used are 1, 2 or 3 per cent sodium bicarbonate, sodium carbonate, sodium borate, or sodium chloride and as much as 1 to 2 pints (0.5 to 1 litre) or more may be given. Three to 4 ounces (90 to 120 ccm) of lime water are sometimes added to each injection, or 5 per cent solution of boric acid may be given by itself. Soap water is a useful vehicle for oil of turpentine or oil of eucalyptus which are added in doses of 1 to 2 drms (4 to 8 ccm) or more. The following are also used:—A decoction made from eucalyptus leaves; 20 drops of benzene added to a pint of warm water or soapsuds; a decoction made by boiling one ounce (30 gm) of chips of quassia in 1½ pints (750 ccm,) of water boiled down to a pint (500 ccm) and strained; 0.4 to 0.5 per cent of quinine solution; 15 to 30 grains (1 to 2 gm) of salicylic acid to a pint of water; ferric chloride 4 drachms (15 ccm) to a pint of water; ferrous sulphate 4 to 10 grains (0.2 to 0.6 gm) in a pint; papain 10 to 20 grains (0.6 to 1.2 gm) in a pint; hydrogen peroxide 1 in 5 to 1 in 10; vinegar diluted with 2 to 3 volumes of water; naphthalene 15 to 30 grains (1 to 2 gm) in 100 ccm of water or 3 ounces of olive oil; tannin in the form of an infusion or decoction of krameria, logwood, catechu, kino and tincture of aloes (drachm to the pint).

A solution of common salt, in the strength of one ounce to a pint of warm water, is quite efficacious for this purpose.

For local application in the perineal region a one per cent ointment of white precipitate may be used to allay the irritation caused by the migration of the worms. In females, the vulva and vagina may be swabbed with one to two per cent phenol and dusted with boric acid or a 5 to 10 per cent boric ointment applied. *Local application to perineum.*

Phenothiazine, a thiazine dye, has been used in the treatment of Enterobiasis, and it is claimed that it gives good and permanent results specially in children. The dosage advised is as follows: 2 gm daily for 7 days for children under 8, half the dose for children under 4; 8 gm daily for at least 5 days for adults; but the drug is very toxic. *Phenothiazine.*

Trichostrongylus is a very minute and slender worm, common in herbivorous animals, especially cattle, sheep and goats, and only occasionally occuring in man. It lives in the duodenum. Its eggs resemble those of hookworms but are larger and more pointed at one end. Infection is thought to occur as in the case of hookworm. Infection is generally symptomless although it is believed that heavy infection may cause severe secondary anaemia. *Trichostrongylus* being very small and easily hidden in mucus and intestinal contents., is not easily reached by anthelmintics, and no reliable treatment is known. *Trichostrongylus.*

A number of drugs have been tried but only gentian violet has proved effective. The drug is given by the mouth in the usual way in tablet form. In the refractary cases it may be given by the duodenal tube. If infection occurs in other organs than intestines, the drug is given by the intravenous route. Faust recommends that after treatment, stool examination should be made for 3 to 6 months by the concentration method, to make sure that cure has been affected. *Treatment.*

TISSUE NEMATODES

(1) Filariasis

The principal human somatic or extra-intestinal parasites, the filariæ, are situated in lymph glands or ducts. Several filariæ occur as human parasites and in some subtropical climates produce various clinical conditions. *Wuchereria bancrofti* is widespread in most tropical countries and also occurs in the temperate regions. The carrier is *Culex fatigans* but other species of mosquito may also carry it. *W. malayi* has also been recorded from areas where *W. bancroft* occurs. *Loa loa* is found in West Africa. *Microfilaria perstans* is found in the blood of people in the West and Central Africa. *Onchocerca volvulus* is prevalent on the Gold Coast and gives rise to localised tumours. This is transmitted by *Simulium damnosum,* a day-biting fly.

Distribution of Filarial infection in India. There are two species of filarial parasites in India, namely, *Wuchereria bancrofti* and *W. malayi.* The former is more widely spread, is characteristically urban in distribution, and is transmitted by *Culex fatigans.* The latter is rural, and is chiefly transmitted by *Mansonoides annulifera.* *Distribution.*

Filariasis is mainly prevalent along the coastal regions and low lying areas near big rivers. A survey of the distribution of this infection reveals that Bengal is the most heavily infected province; Behar, Orissa, United Provinces, Madras and Bombay presidencies coming next in order. The Central Provinces and Nizam's dominion have also isolated areas showing foci of infection, while the Punjab, N. W. Frontier Province, and Rajputana, are practically free. All the hilly regions are free. The central parts of the towns which are densely populated, have a high incidence of infection with *W. bancrofti* while the outlying areas show a low incidence. This confirms the view that the intensity of *W. bancrofti* infection becomes less with a decrease in the density of population.

In Patnagarh (Orissa Feudatory State), Ratanpur, Dhamda (C. P.), and Lakhimpur and Binakandy (Assam), the infection observed was *W. malayi.* The chief transmitter was *Mansonoides annulifera* in all these areas. It was also observed in all these areas, that the outlying wards showed higher infection rates than the central and more densely populated wards. This observation was contrary to what was observed in regard to *W. bancrofti* infection which showed a progressive increase with density of population.

In *W. malayi* area, the types of filarial manifestations were entirely elephantiasis of the legs and elephantiasis of the arms. On the other hand, in *W. bancrofti* areas, besides

elephantiasis of the limbs, a fair proportion of the cases showed genital affections like hydrocoele, elephantiasis of scrotum, penis, vulva and breast, and chyluria.

From a study of the distribution of filarial infection and its variation, the chief factors of endemicity appeared to be (a) the period of effective transmission, (b) suitable breeding places for the transmitting mosquito, and (c) density of population. Temperature and humidity play an important part in the metamorphosis of the filarial embryo in the mosquito. Optimum temperature and humidity must correspond with the breeding of the mosquito and the longer the period of coincidence of these factors the higher the microfilaria-rate.

Transmission.

Transmission of Filarial infection. The chief intermediate host of *W. bancrofti* in nature is *Culex fatigans.* Field observations show that in endemic areas a large percentage of them were found naturally infected. It has been found experimentally that high atmospheric humidity (90 per cent), and a high temperature (80°F), were necessary for the embryo to undergo normal development in the mosquito vector. With this combination of temperature and humidity the rate of development of the worm in the mosquito is very rapid. During the monsoon months when both humidity and temperature are high, the mosquitoes show heavy infection. During this season complete development of the embryos takes seven days. During the winter months the infection rate is very low and the time taken for the completion of development is usually about 18 to 20 days.

Culex fatigans, Anopheles philipinensis, A. pallidus, A. fuliginosus, A. stephensi and *A. ludlowi* were found to be efficient intermediate hosts of *W. bancrofti.* In conformity with the experimental observation, natural infection was observed in *A. ludlowi. A. philipinensis* and *Culexfatigans.*

Pathology.

Pathology:—Adult parasites may not produce any injurious effects and the only indication of their presence may be microfilariae in the blood. They may, however, produce grave lesions. Various stages of inflammatory reaction and fibrosis may occur round the adult parasites. The clinical manifestations due to obstruction of lymphatics and inflammatory reaction are fever. Living or dead worms are frequently found in the glands.

Elephantiasis may be filarial or non-filarial; there may be congenital and sporadic forms of different etiology. *W. bancrofti* is the commonest cause of tropical or endemic elephantiasis. Obstruction of lymphatic vessels and bacterial infection are both important factors in the production of this condition.

Signs and symptoms.

Clinical manifestations:—Fever accompanies lymphangitis and lymphadenitis. The irritation produced by the parasite and subsequent proliferation of the epithelium of the lymphatics, is followed by desquamation. Infection with streptococci and staphylococci commonly occurs, and suppuration and abscess formation may result. Rarely there is complete resolution. The attack of lymphangitis is ushered by a rigor and fever (101 to 104°F) lasting for several days, headache, vomiting, rarely suppression of urine, and albuminuria. The affected part is tender, painful and oedematous; the lymphatics may be hard and cord-like and may show as visible red congested streaks. The skin may be diffusely reddened giving it the appearance of erysipelas. After several days the swelling subsides, and the attack ends with profuse sweating or abscess formation. In other cases the skin may remain infiltrated and attacks may go on recurring, leading to hypertrophy and elephantiasis. Microfilariae may or may not be present in the blood. The inflammatory process may spread to the deep lymphatics.

Elephantoid fever may occur at varying intervals (weeks, months or years) and in India it is popularly associated with lunar influences. Onset of fever is usually quite sudden and the temperature may become normal in 24 hours, or may last several days or even a month. Paroxysms may occur independently of lymphangitis (due probably to inflammation of deep-seated lymphatics not visible). Adenitis of lymph glands may accompany or preceed lymphangitis.

Chyluria.

Funiculitis or lymphangitis of the spermatic cord commonly occurs, and filarial orchitis and hydrocoele are common conditions. Chyluria results from dilatation of the lymphatic duct or some of the abdominal lymphatics communicating with those which carry chyle from the intestines. Rupture of these into the mucous membrane of the urinary tract or bladder, produces chyluria which often starts quite suddenly. Sometime it is preceded by pain in the back, or acting sensation in the lower abdomen and thighs. The attack may be accompanied by fever, and in other cases there may be retention of urine due to coagulated lymph. Chylous ascites may occur. Lymph scrotum, varicose groin glands and filarial abscess may result.

Recent work has brought out the importance of secondary infection in fatal cases of this disease. Many cases of acute septicaemia in endemic areas are due to abdominal filariasis.

Elephantiasis commonly occurs, and in 95 per cent of cases the lower extremeties are involved.

The prognosis, apart from disabilities produced, is good in elephantiasis. The condition is chronic and may last for years.

Rarer complications are filarial synovitis, resulting in fibrotic ankylosis of different joints, or even abscess formation.

Diagnosis. Blood, especially that taken between 10 P. M. and 2 A. M. may show microfilariae. Adult filariae may be found either dead or alive in various localities, microfilaria may be found in chylous urine or hydrocele fluid.

Complement-fixation tests have been carried out with an antigen prepared from adult *Dirofilaria immitis*. It was found that in all cases in which microfilariae were found in the peripheral blood at night, and also in cases of acute lymphangitis, there was a positive reaction. The results of these serological tests support the findings made from the clinical studies. In certain types of cases with an active filarial infection, the clinical symptoms were due to the toxin liberated by the worms. In these cases the complement-fixation tests gave positive results. In others, for example, advanced cases of elephantiasis, where the condition is due to secondary infection, the tests gave negative results, indicating that filarial worms had very little to do with the immediate causation of symptoms in such patients.

A cutaneous test has been devised with 1 per cent saline extract of the *D. immitis* antigen.

Prophylaxis consists in anti-mosquito measures. The microfilaria carrier persons should be isolated so far as possible.

Microfilaria malayi. This microfilaria differs from *W. bancrofti* morphologically and biologically. Intermediate hosts are *Mansonoides annulifera. M. annalatus, Anopheles barbirostres* and *A. hyredvus.* They are mostly night feeders. *W. malayi* is associated with elephantiasis of limbs.

Treatment.

Treatment for filarial infection may be dealt with under the following headings:—

(1) *Anaphylactoid symptoms.* The production of anaphylactic symptoms varies with individual susceptibility. Every case should be carefully investigated and treated accordingly. Recently Goyal and Sundar Rao (1940) have demonstrated the presence of a specific allergen in cases of filariasis and they have also shown that it could be utilized successfully in the treatment of allergic type of cases.

In addition, vaccines made from the various strains of streptococci and staphylococci, and arsenical compounds, such as soamin, have been found to yield good results in the treatment of anaphylactoid signs and symptoms. Heliobrom may control urticarial dermatitis (Manson—Bahr).

(2) *Filarial disease.* Lymphangitis, abscess and elephantiasis are the common manifestations of filarial infection. In a large number of cases the acute attacks are due to secondary infection. When they are caused by streptococci, high temperature sets in with a fairly high leucocyte count. When the infection is in the spermatic cord, it tends to spread towards the peritoneum and sometimes proves fatal. Staphylococcal infection shows milder reaction—the fever is 101° to 102°F. and leucocytosis is not high. Pus is often formed and an abscess appears. For acute signs and symptoms the treatment is symptomatic, and consists of rest, application of ice, diaphoretics, and local application of sedative lotions such as calamine etc.; Sulphadrugs and Penicillin have been

used with good results. In case of abscess formation surgical treatment is necessary.

Lymph varix.

In early stages, application of suspensory or pressure bandages together with treatment for filarial infection is recommended. In advanced cases surgical operation for removal of the varix may be necessary.

In cases of elephantiasis surgical operation for excision of hypertrophied tissue has been recommended. It should be remembered, however, that such operations will not cure the disease. Massage, pressure bandages, prevention of secondary infection and treatment for the same are helpful in reducing the size of the limb and checking its growth. Periodical courses of mixed streptococcal and staphylococcal vaccines have been found to give good results. In case of genital affection, surgical treatment for the excision of elephantoid tissue is recommended.

A large amount of work has been done to find a specific drug for filarial infection, but no success has been attained so far. Many drugs, chiefly compounds of arsenic, antimony, bismuth and copper, and sulphonamides, have been tried without success. Investigations have been carried out with compounds of zinc, lead, tin and gold. Various synthetic and vegetable alkaloids have been tried orally and also by injection, but without success.

Filariasis has been treated with antimony compounds in intensive doses on experimental scale. Workers have tried anthiomaline and some other comparatively newer antimony derivatives, and the results are said to be encouraging.

Prophylaxis of filarial infection consists chiefly in antimosquito measures and isolation of the early-infected cases (carriers).

Chyluria.

Chyluria lymphuria or haemato-chyluria. Blockage by adult parasites at the level of (1) the juxta-aortic glands which drain the lymphatics of the kidneys, or (2) the iliac (internal) glands which drain the lymphatics of the bladder, results in lymph-varix which ruptures on strain or trauma, and leads to leakage of chyle or lymph sometimes mixed with blood. The urine is clear in the morning or after rest but becomes milky after a fatty meal. Cystoscopic and pyelographic examinations are helpful in locating the site and the side affected. Secondary infection is very common in chyluria—culture of a catheter specimen or midstream urine shows streptococci or staphylococci.

Microfilariæ are always present in the blood and urine of patients suffering from chyluria. They are absent in long-standing cases when the parasites are dead. Sometimes they are present only in blood or only in the urine.

For the treament of these conditions absolute rest is necessary. Restriction of fats and fluids is essential. The septic focus or foci should be eradicated and secondary infection treated with autovaccine or stock vaccines (*streptococci* and *staphylococci albus* and *aureus*). Salol, methylene blue, urotropine, picric acid, quinine and potassium iodide have been tried but do not seem to have any specific effect. Astringent injections of Ext. hamamelis liq. or tannic or gallic acid into the bladder give relief. Cautery is helpful in closing the leak in the bladder. Sodium citrate in large doses is given to prevent clotting of chyle in the bladder. The following have been tried with beneficial results:—

Soamin, subcutaneously, intramuscularly or intravenously; commence with 1 gr (0.06 gm) to maximum 3 gr (0.2 gm), twice weekly. Total 20—25 gr (1.3 to 1.6 gm).

N.A.B., neosalvarsan or sulfarsenol, 4—6 doses.

Tryparsamide, 2 gm in 10 ccm of distilled water intravenously once a week, not exceeding a total of 8 gm.

Antimony compounds. Recent observation in human filariasis shows that antimony compounds have no marked effect upon the embryos in the blood though some observers have thought that the number of embryos in the blood shows a marked decrease. Prolonged trials of the antimonyl tartrates in this disease show that the drug has no effect on the adult parasites. Chopra and Sundar Rao (1929) tried almost all the pentavalent organic compounds of antimony, *e.g.*, urea-stibamine, stibosan, neo-stibosan, etc., in filariasis without any effect. The same is the case with other helminthic infestations, *e.g.*, lung infections (paragonmiasis). Recently in the hands of American workers neostibosan has given good results.

Anthiomaline: Brown (1945) used anthiomaline intramuscularly, 3 cc every day till a total of 60 cc was given in 20 days and claimed that microfilaria disappeared from peripheral circulation for about one year. Culbertson and others (1946) used various antimony preparations pentavalent and trivalent with promishing results. They opined that neostibosan I.V. was the best drug when used daily from 0.1 gm rising upto 0.5 gm and using 0.5 gm twice or thrice daily till a total of 7.9-12.5 gm was used. This intensive therapy in the hands of others, was found to be rather too drastic, hence has not been very much approved of.

Hetrazan: Very recently Santiago-stevenson, Hewitt and others (1947) used hetrazan (1-dimethylcarbamyl-4-methylpiperazine hydrochloride) orally on patients harbouring microfilaria of *wuchereria bancrofti* in peripheral blood. The dosage varied from O.5 mgm to 2 mgm. per kilogram body weight three times a day for three to twentytwo days. In all instances the number of microfilarial was markedly reduced from the second day of treatment and in most cases remained low in number for about 8-9 months. Evidence also points to its favourable action on the parent worms.

(2) Loa Loa Filariasis

Loa Loa is a human filaria which occurs largely in West Africa, mainly along the low lying coastal regions and along the course of big rivers and deltaic areas. *Loa Loa* or eye worm, is smaller than *W. bancrofti.* The embryos maintain periodicity in the blood which is exactly the reverse of *W. bancrofti,* that is, the embryos appear in large numbers in the day time and disappear at night. Mangrove fly *Chrysops dimidiata* is the intermediary, development taking place in the thoracic muscles and fat of the body; they pass to the mouth parts and escape in the same way as *W. bancrofti.* After the larvae enter the human body, development is very slow and probably full maturity is not attained till after several years. The worm is long lived. Immature active worms are often seen in children, the embryonic form in the blood is found as a rule in adults and many years after infection.

Loa Loa migrates to the subcutaneous tissues and moves about in this situation; it is frequently found under the skin of fingers, back, and above the sternum, and has a tendency to appear in the ocular region especially the conjunctiva. There is persistent eosinophilia, 30 to 60 per cent. Warmth attracts the worm to the surface of the body.

Clinical features.

The migration of the parasite produces no serious inconvenience but slight local irritation may occur. There may be pricking and creeping sensations and transient œdematous swellings—(calabar swellings) about the size of a hen's egg appear in various parts of the body, in the arms, legs and face; they do not suppurate or cause much trouble. When the parasite appears under the conjunctiva, it may produce considerable irritation and photophobia. If it wanders into the glottis, urethra, etc., it may give rise to serious consequences and great pain. Urticaria and allergic papular dermatitis may sometime occur. Cyst-like swellings may contain adults.

Diagnosis.

Thick blood film may show microfilaria. Confirmatory evidence may be obtained by intra-dermal and complement fixation tests as in case of *W. bancrofti.*

Treatment.

Treatment.—The swelling is very difficult to treat. Use of evaporating lotions relieves irritation. According to some authorities, infection may be controlled by tartar emetic injections, or anthiomaline intravenously (3 ccm of 6 per cent solution every other day). Worms may be removed from under the conjunctiva under local anaesthesia but this does not necessarily remove the infection.

Other filariae.

Other filariae occurring in man are *Mansonella ozzardi* (*F. ozzardi*) which occurs in the West Indies and South America, and is identical with *F. dermarquayi.* The intermediary insects are midges *Culicoides fureus.*

Filaria perstans occurs in the Gold Coast, Central and other parts of Africa and develops in midges *Culicoides austeni.*

(3) Dracontiasis

Dracontiasis is the invasion of the connective tissue by *Dracunculus medinensis,* probably the "fiery serpents" mentioned by Moses. The adult female 30 to 120 cm long and 0.5 to 1 7 mm in diameter. It works its way and arrives at the surface of the skin, producing a local lesion and systemic symptoms, *e.g.,* urticaria, etc. If the parasite ruptures, acute inflammatory symptoms appear. The disease is transmitted by some species of cyclops. When the embryos are swallowed by the cyclops, they pass into the haemocoele cavity where they undergo metamorphosis. In 4 to 5 weeks the larvae mature and are ready to enter the human host.

Life cycle.

The larvae remain in the body of the parent worm and are only freed at the time of parturition, when the head of the worm is brought in contact with water or when it ruptures during extraction. In clear water, the larvae remain alive for six days while in muddy water and moist earth they may live for 2 or 3 weeks. They resuscitate slowly, and sometime only partially.

Transmission.

Transmission.—The cephalic end of the female approaches the skin as the papule formed in the skin becomes vesicular. When the blister ruptures and the part comes in contact with water, the head of the worm is thrust out through the opening in the skin. The uterus of the worm then prolapses through the ruptured anterior wall of the mouth and the first-stage motile larvae are repeatedly discharged into water and are swallowed by the cyclops. Man becomes infected by ingesting the infected cyclops by drinking infected water. The action of gastric juice kills the cyclops and the larval forms become freed. They penetrate the wall of the duodenum or the stomach, migrate through the tissues, and finally lodge in the subcutaneous tissue. It takes 10 to 14 months before the female matures in this position and is ready to discharge its larvae. The male dies after it has fertilized the female and disappears.

Clinical symptoms and pathology.—The female after maturing generally travels towards the legs and feet and sometimes into the scrotum.

Signs and Symptoms.

There may be definite pains in the affected limb and a sensation as if a cord lies under the skin. The symptoms begin a few hours before the parasite nears the surface of the skin. There is also local erythema, generalized urticaria, intense itching, nausea, vomiting, asthma-like symptoms, and giddiness, and even syncope may occur. The symptoms are attributed to toxic secretions from the worm which are given off when it is nearing parturition. The symptoms resemble histamine poisoning and vary according to the susceptibility of the patient. When the head is nearing the surface, there is intense itching, burning, boring or dragging sensation at the spot. The head of the worm usually protrudes through the opening but if not a douche of a stream of cold water is given after which a drop of milky fluid exudes from the head.

The toxic symptoms subside after the rupture of the vesicle and the appearance of the head in the opening. Following this, the area round the worm becomes extremely painful and oedematous, and the toxins excreted and secondary infection may lead to cellulitis. Non-bacterial inflammation has been described to occur round different joints, and is responsible for a lot of crippling. The worm may sometime die before reaching the surface and may become absorbed or calcified. The latter condition may produce synovitis, periostitis or sciatica.

Diagnosis is only possible when the worm appears at the surface of the skin or when it is found on opening an abscess or after aspirating a joint. X—rays may be useful in locating the worm. Eosinophilia about (13 per cent) may be present, but it may be absent. An intradermal test with a saline extract of the parasite is said to be positive in nearly 50 per cent of the cases. *Diagnosis*

Prognosis is good. Generally only one worm is present but sometimes there may be two or more. The danger of reinfection in endemic centres is always present.

Treatment.—As soon as the worm is detected, the patient should be put to bed and the affected part should be rested. Most of the trouble arises because the patient uses the limb, and thus inflammatory changes are favoured, and secondary bacterial infection takes place. When systemic symptoms (urticaria, pruritis, nausea, dyspnoea, etc) are marked, give an intramuscular injection of 5 to 10 minims of adrenaline. The old method of opening the blister with an aseptic scissors, removing the necrotic skin and douching with cold water is still the best. By frequent cold douches, the uterus is gradually forced out and empties the embryos. Until this is done, the hook end of the tail assists the worm to maintain its hold in the lesion. When parturition is completed, and this takes 15 to 20 days, the worm often gets absorbed or tends to come out. The lesion should be kept covered with aseptic gauze and kept moist with sterile water while parturition is taking place. The dressing is changed 2 or 3 times a day and cold douches are given. When the worm emerges, a silk thread is tied to it and it is wrapped round a small piece of bamboo or wood. Attempts should then be made to extract the worm by gentle traction, care being taken not to break it. The average period of extraction is 23 days but it may take as long as ten weeks. Extraction by incision over the worm and dissection is not recommended, except when the worm lies coiled about itself. By surgical extraction, the period of illness may be cut down to 11 days. The worm may be cut out in pieces through the skin at several points, but care should be taken to avoid drawing the anterior end back through the tunnel. *Treatment.*

A number of drugs have been used to kill the parasite, *e.g.*, intravenous injection of tartar emetic or novarsenobenzol; local injections into the worm of chloroform, cocaine, chinosol (1 in 400), perchloride of mercury (1 in 1000) have been tried. The difficulty lies in putting the needle into the body of the worm and even then it is not certain if the fluid will go into it. Injections of 1 in 10,000 acriflavin solution is preferred by some as it produces no local irritation or systemic disturbances and gives immediate relief to itching.

If cellulitis has already occurred, complete rest must be enforced and hot carbolic acid (1 in 40) or lysol fomentations are freely applied. Sulphonamide derivatives, such as sulphathiazole and sulphadiazine may be given in usual doses, penicillin may also be used. If the cellulitis is severe and shows a tendency to spread, incision should be made and pus should be evacuated. Abscesses formed in the breast or in the limbs should be opened and freely drained. *Treatment of cellulitis.*

If joints are affected surgical treatment may be necessary and in most cases removal of sterile pus by aspiration is all that is required.

During convalescence the affected limb should be used with care. Calcified worms may produce sciatica and in such cases removal of these after locating with X-rays may be necessary.

Guinea-worm infection is said to be beneficially affected by intravenous injections of tartar emetic. The worm and the embryos are killed, the inflammation is relieved and as a rule a total of six grains of tartar emetic is said to suffice to

clear the infection, though in some cases a longer course has to be given. Sometimes, the injections have the effect of bringing to the surface other guinea-worms, which happen to be in the body. Hamilton Fairley tried antimony tartrates in guinea-worm infections without success, he prefers emetine injections given intravenously.

Prophylaxis.

Prophylaxis.—Infection occurs by drinking water containing infected cyclops. Stepwells and ponds in villages are source of infection. The larvae should be prevented from reaching the drinking water and cyclops should be destroyed. Cyclops are killed by heating the water 15° to 65°C by passing steam from a boiler, but this treatment has to be frequently repeated and is expensive. Cyclops can also be killed by introducing potassium permanganate or quick lime in solution of 1 in 1,000.

Monthly applications of bleaching powder (3 pounds per 100,000 gallons and copper sulphate 1 pound per 200,000 gallons) kill cyclops.

Biological control by introduction of fish, *Barbus puekelli* which feeds on cyclops and also on the larvae of the worm may be tried. For personal prophylaxis, boiling and filtering of drinking water even through ordinary calico cloth is effective.

(4) Onchocerciasis in Man

Onchoceria volvulus, known as 'blinding worm,' is a tropical nematode widely prevalent in West Africa from Congo to Uganda and Kenya and Sudan. It also occurs in tropical America (Mexico, Columbia, etc.) at an altitude of about 3,000 feet. *O. volvulus* (*F. volvulus*) is found in peculiar sub-cutaneous fibrous multiple tumours varying in size from a pea to a pigeons' egg. Embryos have been found in the blood, but usually microfilariae occur round the tumours which are situated in the regions of the body where lymphatics converge, *e.g.,* axilla, popliteal space, above elbow, sub-occipital region and in the intercostal spaces. The dense mass of connective tissue is mostly made up of coiled bodies of female worms. Usually there are no symptoms, but epileptiform attacks may occur if tumours are present in the scalp and periosteum. Most commonly found in adults of mature age, lymphatic enlargement of the scrotum, hydrocele and enlarged testes may be present, and localised abscess may form. Acute arthritis may occur, and microfilariae may be found in the synovial fluid. Dermatitis, thickening of skin and wrinkling may be present. Lesions of the iris, cornea and choroid may occur, with photophobia, impaired vision, complete opacity (pannus) and blindness.

Treatment.—Excision of tumour in early stages arrests progress of eye or skin symptoms. Intravenous injections of neostibosan sometimes arrest the progress of corneal lesions. Microfilaria may sometime disappear from the skin under intravenous injections of sodium antimony tartrate, continued for four weeks. Protein shock treatment may improve ocular and skin lesions. According to some authorities atebrin in large doses has a definite value. Heliobrom (dibromotannic urea) 10 per cent in spirit applied to the skin at night relieves irritation. Drugs such as gentian violet and hexylresorcinol may be injected into the centre of the nodule to kill adult worms. Injection of 0.1 per cent plasmochin is recommended to control ocular symptoms, by killing worms in the vicinity.

(5) Trichinosis

Trichinella spiralis.

Trichinella spiralis, the trichina worm, is a very small worm parasitic in man, pigs and rats, and occasionally other animals. Man ordinarily becomes infected from eating pork in which the larvae are encysted. When the cysts reach the stomach the larvae are liberated and develop into the adult state in the intestines. The adults live for only a short time in the intestines, reproducing great numbers of living embryos which burrow through the intestinal walls and are carried to various parts of the body by the circulation, and

encyst in striated muscle fibres. This life cycle may occur in any one of the susceptible hosts. The effect of trichinosis depends on the stage of infection, the number of parasites and the locality of encystation. Usually presence of adults in the intestines is not suspected until after reproduction is well under way. Later on such varied symptoms may be produced that the disease may be confused with cholera, dysentery, typhoid, etc. The presence of the cysts in the tissues may cause any symptom and may be confused with any disease. The important point to be remembered, therefore, is that the symptoms lack the regularity of course of any disease. Very little is known about the relative efficacy of different anthelmintics against the reproducing adults, although knowledge on this point is highly desirable. Both the tapeworms and nematode groups of anthelmintics have been recommended. As in the case of strongyloides and trichostrongylus the parasites are easily protected by mucus, etc., and so are not easy to dislodge in large numbers.

Treatment.

There is no specific treatment. The general treatment is the same as of any acute infection. The oral and parenteral use of most of the ordinary anthelimintic drugs is useless and should not be employed. Absolute rest and administration of sedatives, such as aspirin every or 4 hours, will relieve severe muscular pains, or codein may be given in one grain doses. If drugs by the mouth fail, morphine may have to be given. Transfusions of whole blood and plasma may be required. Use of iron by the mouth or by injections may be required.

7. General Considerations in Anthelmintic Drugs

Introduction.

Anthelmintic (from *anti*-against, and *helminthos*-worm) are therapeutic agents used to destroy parasitic helminths in the hosts body or to remove them from the body. It is commonly applied to those drugs which expel worms from the gut but it includes remedies used against worms in the liver, bladder, connective tissue, etc. These remedies have been commonly but inaccurately divided into vermicides (vermes caeders) and vermifuges (vermes fugare). There is, however, no sharp line dividing vermicidal and vermifugal drugs, as very often the same drug may have a vermicidal and a vermifugal action according to the dose in which it is given. Some drugs such as santonin have an entirely vermifugal action as the parasites are expelled alive, while others like chenopodium are vermicidal. Male fern is classed as a vermicide though the tapeworms are often expelled alive. Helminths occuring in blood vessels (*e.g.*, *Schistosoma*) or in the lymphatic vessels, lymph nodes, lymphatic tissues (*Wuchereria*), in the parenchyma of lungs (lung flukes), in the musculature (Trichinella larvae and Cysticerci) may be killed by specific drugs, but the problem of saving the host from the effects of the products of disintegration of the dying or dead worms is yet unsolved.

(1) Requisites of Ideal Anthelmintic

The qualifications requisite for an ideal anthelmintic are:

(1) It should be safe for the patient. It should be borne in mind that anthelmintic drugs, in general, are not toxic specifically to the worms alone, but in a greater or lesser degree, to all living cells; they may even be dangerous to the host. It is, therefore, advisable that prior to the administration of the anthelmintic accurate diagnosis be made by microscopic examinations of the stools, urine, blood, etc., and the patient carefully examined with regard to the condition of his heart, lungs, kidneys and liver so as to be sure that he can safely stand the treatment.

(2) It should be effective in removing the particular kinds of parasites for which it is given. This depends on several factors such as location and

species of parasite, the choice, dosage and purity of the drug used, the preliminary preparation of the patient, administration of a post-purgative, dietary measures, etc. In spite of every precaution there are no anthelmintic drugs which have a 100 per cent efficacy in safe doses for any species of parasites. Fortunately it is not essential to remove all the parasites as unlike bacteria or protozoa, they do not, except in a few instances, multiply in the body. Expulsion of the majority of worms is usually sufficient to render the infection innocuous. It is well known that hookworm infection is practically universally present all over India but the incidence of parasites in certain parts is so low that no untoward effects are produced.

(3) Simplicity of administration. This is of little consequence in the treatment of individual cases, but when treatment has to be carried out in large labour forces it is of great importance, as it not only saves time and trouble but also expense.

(4) The treatment should not be unpleasant and it should have as little after-effects as possible. This is of importance when treating labour forces, as labourers will not take a drug with an unpleasant taste or one which produces disagreeable effects.

(5) The cost of the drug should not be high. No expensive preparation will succeed for use on a large scale as an anthelmintic, however effective it may be.

In the past it has been taken for granted that because a remedy is active against one species of parasite it will be efficacious against others also. These parasites, however, belong to several widely separated zoological groups and differ greatly in their anatomy, physiology, habits and habitat. It is not reasonable, therefore, to expect that any one drug will be effective in anything like an equal degree against such widely different organisms.

From the therapeutic point of view the anthelmintic drugs may be divided into two main groups.

(1) Drugs Acting on Intestinal Helminths

It is desirable to use drugs which are absorbed as little as possible from the gut, thus ensuring their contact with the parasites in maximum concentration and at the same time exerting a minimum effect on the host. The drugs are given by the mouth and recently some have been introduced directly into the duodenum, by a duodenal tube; sometimes drugs are introduced into the rectum and colon in the form of enemata. In rare cases a solution of the drug is used intravenously. Hookworms, roundworms (ascaris) and intestinal flukes are fairly susceptible to these drugs; tapeworms are moderately susceptible, and enterobius, strongyloides, trichostrongylus, trichuris and others still hold out to a large extent against all efforts to dislodge them.

(2) Drugs Acting on Parasites Living in the Tissues of the Body

For the extra-intestinal parasites, readily absorbable drugs are given which are more toxic to the parasites than to the host. These drugs may be given by mouth but are often administered by subcutaneous, intramuscular or intravenous injection. It is only in recent years that any headway has been made against such somatic infections and fairly effective remedies have been discovered for schistosomes, liver flukes, etc.

(3) Modes of Administration

It has already been stated that all drugs belonging to this group are toxic not only to the parasites, but to the host also. Certain precautions regarding diet, dosage and prevention of absorption of the drug are necessary to minimise toxic effects. Anthelmintic treatment consists of three stages:—

Preparation of patient.

(1) *The preparation of the patient.* In the old days starvation of the patient and a preliminary purgative were considered essential, the idea being to get the alimentary canal as empty as possible so that the drug could come into intimate contact with the parasites. It has been found that starvation has, a weakening effect on the patient and renders him more susceptible to the toxic action of the drugs; further, absorption is increased from the empty intestines. In the opinion of some authorities a liquid diet for 24 to 48 hours before treatment is usually preferred with a saline purgative the night before. The withholding of solid food leaves the intestine comparatively empty, so that the drug is less diluted and the preliminary purgative helps to clear away the coat of mucus which covers the parasites. The drug thus comes in contact with the parasites in as high a concentration as possible. Some anthelmintic drugs, such as tetrachlorethylene, carbon tetrachloride and oil of chenopodium are not only quite as effective when given without preliminary starvation and purging, but preliminary fasting and purging considerably enhances their toxicity for the host. For tapeworm infection it is necessary that the drug should come into contact with the heads of the worms, otherwise the main body of the parasite is likely to break off leaving the head intact and in a few weeks the whole chain of segments is regenerated. The preliminary preparation in the form of a liquid diet, consisting of milk or broth on the previous evening, and withholding of food on the day of treatment is, therefore, essential. Even with the most careful preparation the best remedies give only about 50 per cent of cures and, without preparation, the hope of success is much reduced. Strongyloides and trichostrongylus are also deeply imbedded in the intestinal wall and though no specific for them has yet been found the preliminary preparation in the manner mentioned above seems logical.

Absorbable fats are contraindicated with most of the anthelmintics used against the intestinal parasites, as their presence in the gut leads to absorption of these drugs. Many of the anthelmintic drugs, *e.g.*, carbon tetrachloride, thymol, are soluble in alcohol and its presence in the gastro-intestinal tract is liable to increase their absorption, thus producing toxic effects. It is best, therefore, to forbid its use. For remedies which have a deleterious effect upon the liver (*e.g.*, carbon tetrachloride) if this organ is well stocked with glycogen, it is relatively resistant and, therefore, a rich carbohydrate diet is indicated. In the case of drugs which are poisonous but very insoluble in water, *e.g.*, thymol, the amount of water consumed should be cut down to minimum to prevent their solution and absorption. On the other hand, with drugs which are largely absorbed, and excreted by the kidneys, *e.g.*, beta-naphthol, plenty of water should be given.

A limitation of protein diet is said to be harmful to the parasites. Some foods irritate the parasites but this hardly amounts to anthelmintic action. Mechanical irritation with fibrous vegetables, seeds of figs, strawberries, etc., and the husks of grain are said to have unfavourable effect on the worms. Condiments such as mustard, pepper, onion, garlic, spices, etc., were recommended in the olden days as accessories to anthelmintic treatment. Although *in vitro* the anthelmintic power of drugs is enhanced by the presence of these substances, their value *in vivo* is doubtful.

Selection of a preliminary purgative.

The selection of a preliminary purgative depends on the nature of the anthelmintic to be given. In the case of thymol and carbon tetrachloride, oily purgatives should be avoided because these drugs are soluble in fats and the chances of absorption are increased. Non-absorbable oils such as castor oil or paraffin oil are much less dangerous but even these are not recommended. In the case of drugs like chenopodium, oily purgatives can be advantageously used. Chenopodium oil is readily soluble in castor oil and since it is a local irritant its dilution in non-absorbable oils is advantageous in preventing it from coming in contact with the mucous membrane in concentration which will be injurious. Castor oil also neutralises the paralytic action of chenopodium on the intestinal wall. As a general rule, it is preferable to give saline purgatives because they do not increase the solubility of the drug, they create an osmotic pressure and a flow into the lumen of the gut, instead of away from it and lastly they tend to dissolve mucus and, therefore, expose the parasites to the action of the anthelmintic. Magnesium sulphate or sodium sulphate or a combination of the two, in dose of one ounce of the saturated solution diluted to two or three ounces is recommended.

Methods of administration.

The method of administration is determined by the drug prescribed. Water-soluble drugs, if they have not a nauseating taste, may be given in water. Fat soluble drugs, like beta-naphthol and oil of chenopodium, if also water-soluble and absorbable, may be given dissolved in non-absorbable oils. Drugs which are poisonous after absorption in large quantities and which owe their safety to the fact that they are insoluble in water, *e.g.*, thymol, carbon tetrachloride, should under no circumstances be given with fats. It is always safer to avoid the use of oils entirely with male fern. Carbon tetrachloride is insoluble in water and is dangerous if dissolved in oil; it emulsifies when shaken vigorously with milk and the latter forms an excellent medium for its administration. Given in this way it becomes uniformly distributed in the stomach, and the burning sensation which it usually produces is reduced. Tetrachlorethylene is conveniently administered after being shaken up with a solution of the saline purgative in a bottle. For nauseating and objectionable-tasting drugs, *e.g.*, chenopodium, filix mas, etc., gelatine capsules are useful. Hard capsules should always be used since soft gelatine capsules do not dissolve in the upper part of the intestine where the action is desired. Soft capsules are best used in the case of worms residing in the lower portions of the gut, *e.g.*, enterobius, trichuris.

Capsules and Cachets.

Powdered drugs can be given in the form of powders, cachets or tablets and they are often combined with substances which aid their action, *e.g.*, santonin is combined with calomel. It is better to give some drugs such as oil of chenopodium, betanaphthol, male fern, etc., in divided dose at intervals of half an hour to one hour as this insures a longer period of contact with the worms. Besides, if toxic symptoms are produced the next dose can be withheld. Division of doses is, however, disadvantageous when a large number of individuals have to be treated at one time. With drugs like carbon tetrachloride this method is contra-indicated as it is liable to promote absorption.

Duodenal tube.

Drugs introduced directly into the duodenum by Ryles tube are first dissolved in water and slowly run in. In the case of gentian violet solution the tube should be kept for sometime after the solution is run in and then should be withdrawn with caution as the solution, if it comes in contact with the gastric mucosa may set up vomiting.

Enemata.

Anthelmintics in the form of enemata are given for parasites residing in the large intestines and many substances have been recommended for this purpose,

e.g., sodium chloride, quassia, turpentine, hexylresorcinol, etc. The enema should be injected as high up into the colon as possible because often the parasites such as oxyuris are situated in the cæcum and in the neighbourhood of ileocæcal valve. It should be preceded by a purgative and liquid diet the night before, and a preliminary washing out of the colon with soap and hot water on the morning of the treatment. Sometimes an anthelmintic given by the mouth is effective in this class of infection.

Parenteral routes.

For parasites residing in the tissues, intravenous injections of drugs such as tartar emetic, emetine, etc., are desirable; in fasciolopsiasis, emetine has also been used intramuscularly with good results; in paragonimiasis, carpain hydrochloride has been used subcutaneously with success. Anthelmintics may be given by the mouth, *e.g.*, carbon tetrachloride for liver fluke infestations. For lung infestations drugs may be administered by inhalation. Local application of ethyl chloride spray or carbon-dioxide snow or radio therapy is done in creeping eruptions.

Use of post-purgative.

(2) *Expulsion of the worms and the toxic drugs.* A postpurgative may have to be used in the case of some of the anthelmintics as they merely stupefy or paralyse the worms or possibly merely irritate them and if they are not quickly swept out in this condition they may revive and obtain a fresh hold. A brisk purgative in these cases quickly washes them out from the intestines while they are still under the influence of the drug. Some purgatives have an irritating effect on the parasites and the increase of peristaltic movements also helps in removing the unattached parasites. Some anthelmintics have a purgative action of their own, *e.g.*, thymol, hexylresorcinol, and with these, post-purgatives are not necessary though they are often administered. One great advantage of a post-purgative is that it hastens the elimination of the anthelmintic after it has acted on the worms, and in this way absorption and consequent toxic effects, if any, are prevented.

The time at which a post-purgative is given is determined by the location, *e. g.*, for hookworms and ascaris situated in the upper portion of the gut it may be given simultaneously with the anthelmintic (which is an advantage especially in carrying out mass treatments) or very soon after. For tapeworms, situated lower down, the post-purgative is sometimes given one hour after; for enterobius and trichuris an interval of two hours may be necessary. It is often advisable to give another purgative on the second day of treatment, as it may eliminate some of the worms which have succeeded in establishing themselves temporarily in an unfavourable situation lower down in the intestine.

Repetition of courses of treatment especially in case of the intestinal parasites, liver flukes, etc., should never be made in less than one to two weeks, to allow sufficient time for the repair of any damage to the host, caused by the first course. Besides it is seldom possible to be sure that the anthelmintic has failed, in less than a week or even longer. In the case of treatment of tapeworm infection, even when failure is established by unsuccessful search for the head, it is advisable to wait until the parasites have had time to grow again, since the weight of the paralysed or killed worm may draw the head away or may bring it into a position where it is exposed to action of the drug later on.

(4) Criteria of Cure

Disappearance of eggs or embryos in the fæces is the usual criterion of cure and is applicable to all intestinal and hepatic flukes and nematode infections except oxyuris. Eggs deposited in the gut prior to treatment may possibly

appear in the stool for a few days afterwards. Tetrachlorethylene carbon tetrachloride and some other anthelmintics also have a tendency to inhibit production of eggs for a time, without necessarily killing or expelling worms; in the case of tetrachlorethylene and carbon tetrachloride this inhibition may be exerted for as long as ten days. A re-examination of the stool should be carried out after that period, though a positive examination between the third and twelfth day of treatment conclusively indicates failure. Delayed cures are also effected in the case of hookworm and possibly some of the other parasites; the eggs cease to appear and the worms are expelled a few weeks after the administration of the anthelmintic. This is due probably to the parasites being swept into unfavourable positions in the intestine where they persist for some time but eventually lose their hold and are expelled. It should also be borne in mind that reappearance of eggs two to three weeks after treatment, while it shows that a complete cure has not been effected, does not mean that the anthelmintic has proved entirely useless. In the case of hookworms intestinal anthelmintics often cannot reach the young worms which are hidden in the folds of the mucous membrane or between the villi or which have not reached the alimentary tract. The young parasites and even some of the old ones (*e.g.*, strongyloides) may bury themselves in the mucosa, and may thus be out of reach of the drug.

In hookworms.

In enterobius.

In the case of enterobius, the search for eggs cannot be relied upon as a criterion of cure, since eggs are not consistently present in the stools but only when female worms become crushed or disintegrated. The entire stools should be searched for the adult living worms or anal swabs examined for eggs. Besides, reinfection not infrequently occurs and enterobius found in the stools a few weeks after treatment does not necessarily mean that the anthelmintic was ineffective. In strongyloidiasis, the disappearance of larvae from the fæcal smear is not a sure criterion of cure. Repeated cultures of stools as done for growing hookworm larvae, are to be examined, and when these are negative, cure is presumed. In the case of tapeworm the criterion of cure depends on the species involved. The dwarf tapeworm of man (hymenolepsis) can be detected by searching for eggs, but it may be several weeks after treatment that the eggs reappear. This is due to the fact that even though the worms are not expelled the body is broken off just behind the head and it takes time for the segments to re-form. In the majority of tapeworm infections much reliance cannot be placed on searching for eggs; segments may be found in the stool but this takes time. In the case of the tæniæ also the body breaks off and it requires two to three months before the parasite matures and segments reappear.

In tapeworms.

In schistosomes.

In the case of schistosome infection, eggs do not always disappear from the urine and fæces immediately after clinical cure since antimony compounds are lethal both to the eggs as well as to the worms and the eggs which are passed subsequent to the destruction of the adult parasites are dead eggs. Their condition can be determined at once by examining them under the microscope or by the hatching test; this is carried out by washing them and putting them into water when the majority of viable eggs hatch out in a few hours and the liberated embryos can be seen swimming about. In the case of *Wuchereria* infections it is not known how long the embryos circulate in the blood if the parent worms are dead; the criterion of cure has, therefore, to be worked out.

In Filariae.

(5) Experimental Investigation of Anthelmintic drugs

The experimental work is difficult owing to the impossibility of using artificially infected animals on a massive scale as can be done with protozoal diseases. The effect of anthelmintic drugs can be tested on parasites *in vitro,* but these parasites are difficult to obtain and to

keep alive. Sollmann (1918) employed earthworms for testing the action of anthelmintic drugs *in vitro* and found that the drugs which are non-toxic to these worms are not likely to be active, while drugs which are lethal to them have possibilities of effective anthelmintic action. In some cases the toxicity to the earthworm runs parallel with their toxicity to parasitic worms, and a method of assaying these drugs with earthworms has been evolved. The worms are distributed into separate vessels containing carefully measured volumes of liquid; a different amount of the drug to be tested is put in each, except in one vessel which is kept as a control. The behaviour of the worm is then noted. Extreme restlessness or spastic contractions indicate irritation—possibly vermifugal activity. The time of death is noted and the results are compared with a similar series made with a standard preparation. Aspidium, chenopodium, santonin, spigelia, etc., can all be standardised by this method. The use of common earth-worms for *in vitro* experiments is open to obvious objection as they and helminths are wide apart in the Zoological scale. A more rational step would be to collect parasites, human or animal post-mortem.

In vitro tests are, however, merely a preliminary step and must be followed by *in vivo* tests. In most cases dogs respond to anthelmintic drugs in much the same way as human beings though perhaps they are more resistant; they are, therefore, particularly suited for anthelmintic experimentation. As a rule with most of drugs known, a dog weighing 20 kilogrammes can be given the same dose as an average adult human being, but this may not be so in case of all drugs. The worms expelled by the drug are carefully counted and the results are compared with the number of worms still present on post-mortem examination. Darling and Smillie's standard test treatment method is applicable to man. The drug is first given to a series of infected individuals and the worms which are expelled are carefully searched for and counted. Subsequently a full dose of an anthelmintic of known efficacy is administered, and the worms eliminated are compared with those expelled by the experimental drug. All these worm count methods are very laborious and they are not thoroughly reliable. The worms are mostly expelled in the first 18 hours after administration of the drug but elimination may continue for five days and, therefore, all stools during this period should be saved and examined; some worms may even be digested. In veterinary medicine this method has been supplanted in late years by a method of critical testing in which the experimental animals are treated; all worms passed are collected, identified and counted; the animals are then killed and all worms left are collected identified and counted. The figures for worms passed and those present post mortem give a total of those originally present (excepting a few digested ones) and on this total it is possible to express the number passed as a percentage which affords a fairly accurate figure for stating the value of the drug in removing worms.

In vitro tests.

Another method of determining the efficacy of anthelmintic medication is by making differential egg-counts before and after the treatment. In the case of necators for example, the number of eggs produced by a female per day is fairly well known and the number of worms harboured can be roughly estimated by determining the average number of eggs per gram of fæces for a period of not less than three days. Such determinations made before the treatment and then a week or ten days after the treatment enable us to know the relative number of female worms eliminated. In most infections the number of males and females is roughly about equal.

Correlation between chemical composition and anthelmintic action of drugs. The study of the chemical constitution of the active principles isolated from drugs has led to the synthetic production of important compounds with marked physiological properties. Pharmacologists and chemists have been working together to find out what portion of the molecule is responsible for the physiological effects produced by a drug, and this has laid the foundation of the subject of correlation of chemical composition to physiological action of drugs. It is found that though in some cases a distinct relationship can be traced between these two, in the majority of cases the relationship is very obscure and difficult to understand. Very small changes in the chemical constitution completely change the physiological action of drugs, *e.g.*, cocaine has strong local anæsthetic action while alphacocaine has none; l-adrenalin is much more active than d-adrenalin. Changes in the chemical composition of compounds produce changes in their volatility, solubility, osmotic properties and diffusibility, and physiological properties are also altered.

The study of the relationship between the chemical constitution and the anthelmintic action of compounds was first taken up by Hall and Meyer Widgor (1917-18) but their work was interrupted by the War. Caius and Mhaskar made an elaborate study of a large number of anthelmintic drugs and attempted to correlate the efficiency of certain drugs with certain of their chemical constituents. Some very remarkable facts have been brought to light in connection with the anthelmintic action and toxic effects of these drugs:—

(1) Hydrogenation of benzene lowers the toxicity towards the parasites but raises the toxicity towards the host as is the case with menthol.

(2) Total destruction of the benzenoid structure leads to the formation of non-anthelmintic toxic compounds.

(3) Esterification of the phenolic-hydroxyl group leads to the formation of non-anthelmintic non-toxic compounds, *e. g.,* thymotal.

(4) The mere presence of the phenolic-hydroxyl group does not always confer anthelmintic properties, *e.g.,* iso-amyl-phenol or iso-butyl-phenol which contain it are both inactive.

(5) The active anthelmintic principles in oil of chenopodium (paracymene, phellandrene, ascaridol) and thymol, contain free phenolic hydroxyl to which are attributed their anthelmintic properties.

(6) Methyl alkyl appears to intensify the anthelmintic action of phenolic-hydroxyl, *e. g.,* methyl-salicylate has marked anthelmintic properties while other salicylic acid compounds such as aspirin and salol are inert.

(7) Neither alcohol nor aldehyde groups have any anthelmintic properties.
(8) The anthelmintic activity of carbon compounds appears to be correlated with the chlorine content.

It will be seen, therefore, that in the majority of cases the relation between chemical constitution and their physiological action is not very clear. Change in chemical composition completely changes the physical properties of compounds. Their volatility, solubility, osmotic properties, ionisation, etc., undergo a complete change and so their absorption into the system and the physiological effects produced are also modified. In the present state of our knowledge of the changes going on inside the body of a living cell, it is impossible to speculate further.

8. Drugs Used Against Helminths

The following is a list of the more common present-day chemotherapeutic substances used as anthelmintics and adjuvants (the latter are marked by asterisks), arranged by Faust (1939) according to chemical nature, to which recently discovered anthelmintics have been added.

I. INORGANIC

*Calomel
Carbon-dioxide snow
Colloidal antimony
*Ferrous carbonate (in Blaud's pills)
*Magnesium sulphate
*Sodium sulphate

II. ORGANIC

1. Hydrocarbons
   *Mineral oils
2. Aliphatic halogenated hydrocarbons
   Carbon tetrachloride
   Chloroform
   Ethyl chloride
   Tetrachlorethylene
3. Phenols
   B-naphthol
   Hexylresorcinol
   Neoantimosan
   Thymol
4. Terpenes
   Ascaridol
   Santonin

5. Triamino triphenyl methane
   Gentian violet
6. Organic acids, their salts and esters
   Antimony and potassium tartrate (tartar emetic)
   *Calcium gluconate
   *Calcium lactate
   *Iron and ammonium citrate
   Sodium antimonyl tartrate
7. Piperazine derivatives (Hetrazan)
8. Alkaloids
   Arecoline (in areca or betel nut)
   Emetine hydrochloride
   Margosine
   Pelletierine
   Pyrethrins
9. Enzymes
   Papain
   Filicin (in leche de higueron)
10. Semi-refined or unrefined plant products
    Areca or betel nut
    Aspidium, oleresin
    *Castor oil
    Cocoanut
    Corsican moss (helmintocorton)
    Garlic
    Granatum (pomegranate)
    Kamala
    Kousso
    Leche de higueron
    Oleum chenopodii
    Oleum meliae (azedarach)
    Onion
    Papaya
    Pepo (pumpkin seed)
    Pyrethrum
    Quassia

(1) Male Fern and Allied Drugs

Male fern, Kousso and Kamala are the three important drugs of this group. Though derived from different sources they are intimately related to each other from a chemical as well as from a pharmacological point of view. The active principles in all are chemically allied bodies, *i.e.*, derivatives of phloroglucin.

(a) Aspidium (Male Fern)

Synonyms:—Stipides aspidii, Radix Filicis Maris, Sweet brake.

Male fern.

This is one of the oldest anthelmintic drugs known and was used by the ancient physicians, Pliny and Galen. *Aspidium filix mas* and *Aspidium marginalis* are the two well-known varieties of ferns which are recognised in the British and United States Pharmacopœias. They both contain the same active principles, but the former yields smaller quantities of the rhizome. Several other forms such as *A. spinulosum* and *A. dilatatun* growing in Europe and *A. anthelminticum* growing in South America contain similar active principles and properties. The rhizome and stipes of *Dryopteris filix mas* (Linn.) Schott (Fam. Polypodiaceæ) are chiefly used.

Chemical composition.

The chemical constituents of the rhizome are unstable bodies and undergo chemical changes even in the dry rhizome. The active principles are a number of non-nitrogenous substances the more important of which are as follows:—*filmaron* 5 per cent, *filicic acid* 2 to 4 per cent, and smaller quantities of aspidin, aspidinol and filicic acid. In addition there are tannic acid 10 per cent; inactive filicic anhydride (filicin) produced in old specimens by transformation of filicic acid, 19 to 31 per cent; a green fixed oil 6 to 7 per

cent; a volatile oil 0.02 to 0.04 per cent; an uncrystallisable sugar, 11 per cent; resin, starch, and wax.

The active substances are compounds of butyric and isobutyric acids with phloroglucin and its homologues. They are insoluble in water and soluble in fats and oils. The essential oil is light yellow in colour and gives it its peculiar odour. The poor absorption from the gut is dependent on the insolubility of the active principles in water.

Filicic acid or *filicin* occurs in two forms, (1) a crystalline inactive form and (2) an amorphous form which is said to be mainly responsible for the therapeutic activity of the drug. The crystalline form is the lactone of the active amorphous form and the latter readily changes into the former. This is the reason why the rhizome and its preparations deteriorate so quickly.

Pharmacological action.

Straub has shown that the active principles of male fern are powerful poisons to smooth muscle and they also attack striated muscle. This quality of the drug probably accounts for its vermicidal action on the tapeworm. *In vitro* experiments show that non-striated muscle of invertebrates such as molluscs, annelids, echinoderms, crustaceans, etc., is very sensitive to filicic acid bodies and is rapidly paralysed by its direct action on the muscle fibres.

Internally, filicic acid has a strongly irritant action on the gastro-intestinal tract and may produce vomiting and blood-stained diarrhœa, and in excessive doses collapse and death. The irritation of the gastro-intestinal tract sets up reflex uterine contractions. It should not, therefore, be given to pregnant women. The liver is affected even after moderate dose producing bilirubinæmia, large doses may produce jaundice and cirrhosis of the liver. The heart muscle is depressed and the beats are slowed and weakened. The spinal cord is stimulated, at first producing twitchings and with large doses convulsions occur; later, ascending paralysis of the spinal cord sets in, and death occurs from failure of the respiratory centre. Small amounts of the active principles are absorbed from the gut and filicic acid and its decomposition products are excreted by the kidneys.

Principles of administration of Filix mas.

The fresh ethereal extract is usually administered in dosage of 10 to 30 minims every half an hour in capsules till the full dose is given; a grain of calomel may be added to each capsule. It may also be given in an ounce of milk with the yolk of an egg or some flavoring agent such as cinnamon oil or chloroform water, to conceal its nauseating taste. Two to 10 minims (0.1 to 0.6 ccm) of chloroform are said to enhance its efficacy. The oleoresin is given in a 30 grain dose (2 gm) to an adult and 7½ to 15 grains (0.5 to 1.0 gm) for a child. It is best given in divided doses 10 to 30 minutes apart, the last dose being followed in ½ to 1 hour by a full dose of magnesium sulphate. Pills coated with keratin are preferred by some. Clayton Lane and Low recommend the following method of administration of the drug: liquid diet for two days; calomel or a saline purge in the afternoon of the second day; a cup of black coffee the next morning (third day); then one capsule containing 30 minims of the liquid extract at intervals of ½ hour till three such are given. A drachm of brandy with 15 minims (1.0 ccm) of chloroform may be given with the first dose, to prevent vomiting. A saline aperient (Epsom salts, Carlsbad salts or sodium sulphate) is given two hours after the last dose, to wash out the parasites and the drug.

In treating cases with the extract of male fern, three things are essential; (1) The alimentary canal should be properly prepared for the reception of the drug. The head of the parasite lies imbedded in the intestinal mucosa between the villi, and unless the bowel is empty the drug does not reach it. (2) The extract, if used, should be fresh. This is important as the power of the extract to retain its potency is uncertain. The active principles of filix mas are easily destroyed and it has been noticed by several observers that the dried rhizome of the male fern gradually loses its activity on keeping. The extract should be made from specimens that have not been kept for more than a year. (3) The post-

purgative given should be a powerful one, so that the head of the worm loses its hold and is expelled from the intestine.

All stools passed for 24 hours after treatment should be saved and passed through a fine sieve to search for the presence of the scolex or the head. If the head is not found, cure has probably not been effected and another course of treatment will be necessary. If the major portion of the worm has not been expelled treatment might be repeated after an interval of 10 to 15 days following the first course, as the drug undoubtedly has a cumulative toxic effect. The possibility of there being more than one worm should be borne in mind and this can be usually determined by piecing together the expelled segments Some authorities administer this treatment, by means of duodenal tube, thus preventing the chance of vomiting out the drug.

Anthelmintic effects.

The older physicians used this drug against all forms of helminthic infections but recent work has shown that it has little value against ascaris, hookworms and other nematodes. Against whipworms (trichuris) a small dose of the extract (1½ drachm) daily 2 to 3 days in succession and repeated after an interval of 10 days is effective. It is useless in schistosomiasis, somatic tæniasis, etc., although the drug is said to have some effect against liver flukes. On the cestodes the drug has almost a specific effect though failures are not uncommon. The failures recorded are mostly due to the poor quality of the drug obtained from the market and the careless preparation of the patient. Rarely, the parasites themselves show extraordinary resistance to the drug and cannot be expelled even after 5 or 6 treatments.

In Europe and America, *filix mas* is commonly used against *Taenia* or *Diphyllobothrium*. If the patients are properly prepared according to Lane and Low's method, 50 per cent of cases of *Taenia saginata* infections are cured with a single treatment. It acts on *Hymenolepis nana* as well as the large tænias, but as the former are small and are embedded in the mucus it is difficult for the drug to reach them. The rarer tapeworms of man, *e.g., Dipylidium caninum, Hymenolepis diminuta* and *Bertiella studeri* which are not normal human parasites, are more easily expelled than the species which have adapted themselves to the human host.

Signs and symptoms of poisoning.

Male fern was formerly considered to be a harmless drug in man but recent experience has shown that it is very toxic and deaths have been reported from its use. Toxic doses vary within wide limits depending on the active principles present and also on the resistance of the patient. Anæmia, debility, old age and infancy are predisposing factors to poisoning. The presence of fatty substances in the gut increases absorption. Toxic symptoms are likely to occur after 2 to 2½ drachms (8 to 10 gm) of the extract, but severe poisoning has occurred after 1 drachm (4 gm) given in a day. More than 2 drachms should never be given in 24 hours.

The chief symptoms in these cases are headache, vertigo and bilirubinæmia; slight jaundice frequently occurs. In moderately severe doses the symptoms are those of gastro-enteritis, *e.g.,* vomiting, abdominal pain, blood-stained diarrhœa; headache, dizziness, shortness of breath, dimness of vision or yellow vision and sometimes amblyopia may be present. The patient becomes dyspnœic, looks drowsy and feeble. The kidneys are irritated and albumin is often present in the urine. Hæmoglobinuria and hæmaturia rarely occur. In very severe cases the patient becomes delirious, gets severe cramps and violent convulsions; he loses consciousness, respiration becomes slow and shallow, the pulse weak

and imperceptible and there is marked cyanosis. Convulsions are followed by muscular relaxation, immobility of pupil, and abolition of reflexes are common. Optic neuritis resulting in temporary or even permanent blindness has been observed. Death occurs from respiratory failure or paralysis of the heart.

Treatment of poisoning.

In treating cases of male fern poisoning, the stomach and the intestines should be evacuated at once. A quickly acting purgative such as magnesium sulphate or citrate may be given at once. Such purgatives as castor oil or croton oil should be avoided. Demulcent drinks should be given if diarrhœa and vomiting are present. The patient should be kept strictly in a recumbent posture, for there is danger of cardiac failure, and he should be kept warm. Cardiac stimulants, such as aromatic spirit of ammonia, spirit of ether or camphorated oil should be administered. If collapse occurs, give injections of pituitrin, adrenalin or ephedrine. Convulsions if present should be controlled by inhalation of chloroform or ether; bromides may be given. If paralysis is present, give atropine or strychnine. Alkalies may be administered if hæmaturia is present.

Precautions and contra-indications.

Preliminary preparatory measures should not be carried to extremes and starvation is not indicated; the patient should be given a light diet and a preliminary purge. The administration of the extract should be followed up by a strong active purgative, so that it is eliminated from the gut without delay. Under no circumstances oily cathartics like castor oil, which facilitate absorption of the drug, should be given, for in 57 per cent of cases severe poisoning has occurred subsequent to its administration. Other solvents such as fats and alcohol should be avoided. A rapidly acting purgative such as magnesium sulphate, should be administered. Compound jalap powder, senna or calomel may be given previously or along with the drug. Weak and emaciated patients are not suitable cases for the administration of the drug. The drug is contra-indicated when ulceration of the stomach and duodenum is present because of its irritant action and the liability of greater absorption from the ulcerated surface. It should also not be given in pregnancy, affections of the heart, liver and kidneys.

Preparations.

Extractum filicis liquidum (B. P.) is prepared by ether extraction and contains not less than 20 per cent of filicin. Dose 45 to 90 minims (3 to 6 ccm); as much as 2 drachms may be given. The extract loses its activity on keeping. The specimen of a good active extract is dark green in colour, while an extract which has turned brownish yellow and has a crystalline deposit at the bottom has deteriorated. As the extract has a very disagreeable taste and smell it is often given in capsules containing 15 minims each. Yeo gives capsules with one grain of calomel each. *Haustus filicis liq.* is in use in many hospitals. It consists of liquid extract of filix mas one drachm, syrup of ginger one drachm, tincture of quillaia ½ drachm and peppermint water up to one ounce.

Oleoresina aspidium (U.S.P.) made by percolation with ether, is a solid substance and does not deteriorate so rapidly as the liquid extract. Dose 30 grains (2.0 gm) once a day.

Filmaron is soluble in ether, alkali, and sparingly in alcohol. Dose 10 to 15 grains (0.6 to 1.0 gm).

Amorphous filicic acid. Dose 8 to 15 grains (0.5 to 1.0 gm) in a capsule.

(b) Kousso or Cusso

Kousso is the dried female flowers of *Hagenia abyssinica* also known as *Brayera anthelmintica,* family Rosaceæ. The plant grows in north-eastern part of Africa and is cultivated in Abyssinia. It has been used as an anthelmintic from very ancient times. The entire panicles of the pistillate flowers collected after pollination, and dried in the sun are most suitable for vermicidal purposes. Only the panicles are official in the pharmacopœia.

Kousso has no odour but has a bitter acrid taste. The drug loses its activity by ageing and, therefore, should be used as fresh as possible.

Chemical composition.

Kousso is composed of two distinct parts: (1) the glands of the flower panicles containing the active principles and (2) sharp microscopic hairs which are mechanically irritating to worms and to the gut. The active constituent of kousso is *kosotoxin* which is a pale yellow, amorphous powder, insoluble in water but soluble in alcohol and ether; it melts at 80°C. Besides these it contains a votatile oil, resin, tannic acid, oil and gum. The commercial *koussein* is a mixture of various principles.

Pharmacological action.

Kosotoxin is a protoplasmic poison. In frogs it acts like curare and paralyses the motor nerve endings of the striped muscle and finally the heart. In mammals, it stimulates the medullary centres. Both tapeworms and ascaris die when exposed for 30 minutes to an infusion of kousso.

Therapeutic uses.

Kousso is used as a tæniafuge and is said to be the safest among this class of drugs. Its efficacy depends on its freshness. It is equally effective against all species of tapeworms and has also been used against ascaris. Koussein in small doses is preferable to other preparations. Usually 15 to 60 grains (1 to 4 gm) are given in a capsule in divided doses hourly or half hourly, in much the same way as the oleoresin of malefern. The last dose is followed by a purgative. The usual dose for an adult is 2 to 4 drachms (4 to 8 gm) of the powdered flowers. No fasting on the previous day is neccessary. The drug is given in the morning on an empty stomach after the bowels have been moved, and no food except clear soup or weak tea is given till the worms are expelled. The infusion may be given in doses of 8 ounces (250 ccm) at once, or divided into 2 or 3 portions. As a rule, no after purgative is necessary, but if the bowels do not move a saline purgative should be given. Kousso is both a tæniacide and a tæniafuge, the latter property is dependent partly on the purgative action of the active principle and partly on the numerous fine pointed hairs which irritate the worm and drive it out.

Toxic effects.

Kosotoxin is a toxic drug, 1 mgm being fatal in a frog and 50 mgm per kilo in rabbits, dogs and cats. The lethal dose in man is not known. Moderately large doses produce nausea, vomiting and faintness; purgation usually begins after an hour. Larger doses produce gastro-intestinal irritation with continued vomiting and violent purgation. If the drug is absorbed there is headache, vertigo, mental disturbance, precordial pain, dyspnœa, painful micturition and sometimes convulsions.

Kousso and its preparations are contra-indicated in pregnancy, debility, and organic diseases of the heart and kidneys.

Preparations.

Powdered dried panicles of the pistillate flower are given in doses of 2 to 4 drachms (8 to 16 gm)

Kosin (N. O.) is a yellowish bitter crystalline mass. Dose 7 to 15 grains (0.45 to 1.0 gm) repeated every half hour till 4 doses have been given; last dose followed by a purgative.

Infusion brayerae or infusion of kousso is prepared by pouring 1,000 ccm of boiling water over 50 gm of powdered brayer and allowing it to stand till cool. It is given without straining. Dose 6 to 10 ounces (190 to 300 ccm).

(c) Kamala

SYNONYMS:—ROTTLERA, KAMEELA, REROO.

Kamala is a purplish-red or brick-red powder derived from the glands and hair of the fruit of *Mallotus philippinensis*, an evergreen tree belonging to the Euphorbiaceæ or spurge family. It has long been used as a tæniacide in India and at one time had gained a considerable reputation as an anthelmintic and was included in the British and United States Pharmacopœias. Further experience, however, showed that its action was uncertain and it was, therefore, discarded.

Active principles consist of:—(1) a brownish-red resin or resinoid *rottlerin* (mallotoxin) It is probably the same as *kamaline*. (2) *Iso-rottlerin* is probably impure rottlerin. Besides these, there are resins, starch, traces of a volatile oil, oxalic and citric acids. Powdered kamala is given in doses of 2 to 3 drachms mixed with milk, curds or honey. The drug

met with on the market is often adulterated with ferruginous sand, red brick dust, ferric, oxide, dyed starch, etc.

Action and uses.

The drug irritates the gastro-intestinal tract and even in therapeutic doses produces considerable nausea and increase of peristaltic movements of the gut. It has thus a purgative action. Large doses produce gastro-intestinal irritation and set up vomiting and violent purging.

The chief advantage of this drug appears to be that it can be safely given without any preliminary preparation of the patient; no post-purgative is necessary. It is much less efficient against tapeworm than male fern. It is useless against hookworm, ascaris and whipworm. It is a mild drug and is only indicated in children and debilitated individuals in whom extract of filix mas is not advisable.

(d) Other Anthelmintics

POMEGRANATE OR GRANATUM

Pomegranate.

The bark of the pomegranate tree, *Punica granatum* Linn. (N. O. Lythraceæ), has been employed as an anthelmintic in India. Both the root bark and the stem bark have been used but root bark is preferable inasmuch as the alkaloidal content is greater than that of the stem bark.

Chemical composition.

The active principles of the bark are 4 alkaloids: *Pelletierine* or *punicine,* the most important of the group,, is a colourless volatile liquid which turns dark on exposure and *Iso-pelletierine.* Only these two alkaloids are supposed to possess activity against tapeworms. *Methyl-pelletierine* closely resembles pelletierine and *Pseudo-pelletierine* also known as *methyl granatine* are inactive. The mixed alkaloids were put on the market under the name of pelletierine (the names punicine, isopunicine, etc., have been adopted for the pure alkaloids). The bark also contains *puni-cotannic acid* which has a tæniafugal action, and it renders the alkaloids insoluble and prevents their absorption from the gut. The sulphates of the alkaloids are soluble and are readily absorbed. This explains why the bark is a better anthelmintic.

Pharmacological action.

The alkaloids pelletierine and iso-pelletierine have a local irritant action and even in small doses they produce vomiting and colicky diarrhoea. They are absorbed from the gut if given in soluble form and are readily destroyed in the body. They act on the nervous system producing headache, vertigo, drowsiness, ocular disturbances and dimness of vision. Toxic doses have a paralysing effect on the motor nerve endings and give rise to numbness and cramps of the lower extremities, paralysis of the diaphragm and convulsions from asphyxia. In rabbits there is progressive muscular paralysis. Unstriped muscle fibres are first stimulated then paralysed. *Taenia saginata* which usually live for hours in a solution of sodium chloride kept at body temperature, are killed in about 10 minutes if 0.01 per cent pelletierine is added to the solution.

Anthelmintic use.

The fresh bark is said to be a fairly good anthelmintic against tapeworms. The bark is, however, unpleasant to take and on account of the large quantities of tannic acid present it is liable to produce nausea and vomiting. Experience shows that it is also uncertain in its action. Pelletierine in the form of tannate given in doses of 2 to 8 grains (0.12 to 0.5gm) acts as an excellent tæniacide without producing marked toxic effects. The soluble salts are absorbed and produce toxic effects. Absorption is favoured by alcohol, and for this reason fluid extract is not such a good tæniafuge as the decoction or powdered bark. If the preliminary preparation of the patient by dieting and purging is carefully attended to in the same way as with aspidium, pelletierine tannate will seldom fail to produce an efficient vermicidal action. After liquid diet for two days and a strong preliminary saline purgative the night before treatment, pelletierine is given in the morning in sweetened water followed by a brisk saline cathartic half an hour later. The patient should stay in bed after taking the medicine as dizziness is always experienced. The worms are expelled 2 to 4 hours later. This drug ranks next only to filix mas as a tæniacide, and is specially recommended against infestations with *Taenia solium.* Against *Hymenolepis nana* it is not effective.

Toxic effects.

Large doses of the decoction or the alkaloids produce headache, dizziness, dim vision, nausea, vomiting, diarrhœa, weakness of the limbs followed by paralysis. Drowsiness and coma may supervene. Serious disturbances in the eyeball such as dilatation of the pupil, retinal congestion and amaurosis may occasionally occur.

Treatment is largely symptomatic; rapid emptying of the bowels, administration of stimulants and artificial respiration when the respiratory muscles are affected, are important. A brisk purgative such as calomel and saline should be given immediately. If the patient is seen soon after ingestion of a large amount of the drug, gastric lavage is indicated. Demulcent drinks to soothe the gastro-intestinal irritation are recommended. Tannic acid may be given. Stimulants such as strychnine are indicated. *Treatment of poisoning.*

The dried powdered bark is given in doses of 20 to 40 grains (1.3 to 2.0 gm.). *Preparations.*

Granati (U.S.P.X.) is prepared by exhausting the powdered bark with 37 per cent alcohol and 10 per cent glycerine. Each ccm represents 1.0 gm of the drug.

Pelletierine (mixture of the alkaloids), dose 2 to 6 grains. *Ext. Granati Liq.,* dose 30 to 120 minims. *Pelletierine sulphate,* dose 2 to 3 grains. *Pelletierine tannate* (B. P.) is a yellow, amorphous powder, dose 2 to 8 grains. *Pelletierine hydrobromide* is a brownish viscid liquid, dose 2 to 8 minims.

ARECA NUT. Synonyms:—arecæ semina, betel nut. Areca nut is the seed of *Areca catechu* belonging to the family Palmaceæ. It has been used for a long time in India and China as an anthelmintic both for man and animals and was considered so effective that it was included in the British Pharmacopœia. Recent work has thrown considerable doubt on its anthelmintic value.

Several alkaloids have been isolated from the seeds which are all chemically related to each other. These are, *arecoline* and *arecaine* which occur in the same proportions (0.1 per cent), *arecaidine* and *guvacine* occur only in traces. *Arecoline,* a colourless oily liquid is physiologically active; the other three are probably inactive. To it the anthelmintic and sialogogue properties are mainly to be attributed. There is also an essential oil (which contains laurin and myristin), several sugars, iron, magnesium and other salts. *Chemical composition.*

Arecoline base is not soluble in water and is not readily absorbed; the soluble salts of arecoline are rapidly taken up from all mucous membranes and poisoning can be produred by instilling a 1.0 per cent solution into the eye. It is excreted unchanged in the urine. The action of arecoline resembles that of pilocarpine and physostigmine and is chiefly on the autonomic and central nervous systems. It stimulates the nerve endings of the plain muscles and glands; there is inhibition of the heart and fall of blood pressure, constriction of the pupil, contraction of the bronchial musculature and definite increase of the peristaltic movements of the intestine owing to stimulation of Auerbach's plexus; the flow of saliva and sweat is stimulated. Tapeworms and liver flukes placed in dilute solutions of arecoline salts are rapidly paralysed. *Pharmacological action of arecoline.*

Areca nut is not an effective drug against nematodes and its efficacy against tapeworms and flukes is doubtful. The powdered nut in doses of 1 to 3 drachms (4 to 12 gm) is given in syrup. It has undoubtedly an irritant action on the gut and produces purgation, but it does not expel worms. Arecoline salts are given by the mouth in man in doses of 1/15 to 1/10 grain (4 to 6 mgm) or subcutaneously ¼ of the amount. One to 2 grains are given to cattle to relieve colic. *Anthelmintic uses.*

Arecoline in pure condition is a toxic drug. As it is readily absorbed from the gastro-intestinal tract it is considered dangerous for use as a tæniacide. Arecoline poisoning, however, might occur when large doses of the powdered nuts are administered. The symptoms resemble those of fungus poisoning (muscarine) and consist of vomiting, diarrhœa, colicky pains, marked salivation, laboured breathing, tremors, convulsions, coma and death. If toxic symptoms supervene, rapid evacuation of the bowels and administration of cardiac and respiratory stimulants are indicated. *Toxic effects.*

Dry and fresh powdered seeds, dose 1 to 3 drachms (4 to 12 gm). *Preparation.*

Arecoline dihydrobromide and *dihydrochloride* occur in white crystals soluble in alcohol and in water. It is official in some of the pharmacopœias, dose 1/120 to 1/40 grain.

PEPO. Pumpkin seeds. The seeds of *Cucurbita pepo* and *C. maxima* (melon pumpkin) have been used for ages as a household remedy against tapeworms. They are said to have succeeded when other drugs have failed. There are no active principles, and the therapeutic activity, if any, is probably due to mechanical action of the sharp edges of the bruised seeds.

C. pepo contains 40 per cent of a fixed oil and 30 per cent of starch, a soft bitter acrid resin which is said to be the active constituent, a volatile oil, and 30 per cent of starch, ***Chemical composition.***

Pharmacological action.

sugars and proteins. *C. maxima* contains 30 per cent of a fixed oil and an acid resin, which was supposed to be responsible for the anthelmintic activity of the seeds but later proved to be useless. *Ascaris, Taenia saginata* and *Diphyllobothrium latum* are said to be irritated and dislodged by the husks and paralysed by the oil and resin. *Taenia solium* and *Hymenolepis nana* are apparently more resistant.

Anthelmintic effects.

The seeds administered should be fresh, certainly not more than a month old. Two to four ounces are administered crushed or beaten into a paste with water and finely divided sugar, the whole being made up to one pint. Sometimes a little milk is added. The usual preparatory dietetic measures are carried out and a saline purgative is given the night before and again early the following morning. A very light breakfast is taken and two hours later the seeds are given in three portions, 2 hours apart. The patient should remain in bed. If effective purgation does not occur within 3 hours of administration, a dose of castor oil should be given. Experiments recently carried out on dogs to test the anthelmintic effects of the seeds gave inconclusive results. The seeds are sometimes taken three or four times a day for a few days until the worms are expelled. Symptoms are produced even after large doses.

Preparations.

Cucurbita semina praeparata (B. P.). Dose 1 to 2 ounces (30 to 60 gm) or more.

Cocoanut.

Cocoanut. The flesh and milk of the cocoanut, the fruit of a palm *Cocos nucifera* are said to have anthelmintic properties and to be specially effective against tapeworms. They have been largely used in India and other Eastern countries. When ingested in large quantities, the milk and the meat have a purgative action and are said to expel not only the worm but its head also. This, however, is doubtful. The patient usually fasts for 24 hours, drinks the milk and eats the meat of one whole nut; 2 to 3 hours later a purgative is given.

Musenna.

The bark of *Acacia anthelmintica* or *Albizzia anthelmintica,* an Abyssinian tree belonging to N. O. Leguminosæ, is used as a tæniacide in Abyssinia. The bark is sold in flat pieces 5 to 10 inches long and is pale yellow in colour. It has acrid taste and contains resins, an amorphous saponin-like principle *musennin* and an alkaloid *moussenine.* Two to four ounces of the powdered bark suspended in water or mixed with flour in the form of bread, or made into an electuary with honey are taken in the morning before breakfast. In Western medicine musenna is used in the form of an infusion in doses of 1 to 6 ounces, given in several portions and followed by a purgative. Moussenine has been isolated and given in doses of 3 to 4 grains (0.2 to 0.25 gm).

(2) Carbon Derivatives

This series of compounds is composed mainly of carbon and halogens. It was first suggested that anthelmintic activity is correlated with the chlorine content and since chloroform shows a considerable anthelmintic efficacy against hookworm, some of the related compounds with higher halogen content may prove even more effective. Working on this hypothesis carbon tetrachloride (CCl_4) was tried as an anthelmintic with excellent results. Carbon tetrachloride has a marked anthelmintic effect against hookworms and its toxicity to animals is not very great. Owing to its low solubility and volatility and consequent slow rate of diffusion, comparatively small quantities are absorbed into the circulation. Large quantities can, therefore, be introduced into the alimentary canal without untoward effects.

Cases are, however, on record where alarming symptoms develop from therapeutic doses either due to idiosyncracy or weak condition of the patient associated with a diseased liver. Other compounds related to carbon tetrachloride have also been tried. Ethylene dichloride or dichlorethylene ($C_2H_2Cl_2$) has been shown to be an effective anthelmintic against hookworms but it is distinctly weaker than chloroform and carbon tetrachloride; tetrachlor-ethylene (C_2Cl_4) is as effective against hookworms as carbon tetrachloride. Recent work by Maplestone and Chopra (1933) on the toxicity of tetra-chlorethylene shows that in comparison with carbon tetrachloride, its toxicity is very low and use of alcohol does not seem to be a contraindication. All these advantages make it the drug of choice.

Carbon trichloride or hexachlorethane (C_2Cl_6) is another of these compounds which has been tested but found ineffective. It is difficult to say, which are the factors responsible for the anthelmintic properties of these compounds. The chlorine content or chlorine

concentration has been suggested as the explanation but a close study of the properties of these compounds seems to point to the physical factor of solubility.

(a) Tetrachlorethylene

Tetrachlorethylene.

It is also known as perchlorethylene or carbon dichloride. It is an unsaturated chlorinated aliphatic hydrocarbon, having the formula, C_2Cl_4. It is a heavy liquid, having a specific gravity of 1.6, boiling point varying between 110°-120°C and chlorine content of 85.5 per cent. It is very insoluble in water (1 in 10,000 or less). The drug is fairly stable in all climatic conditions, but it should be kept away from the air in well-stoppered amber bottles.

Pharmacological action.

It is non-irritating and does not produce any local effect on the mucous membrane. Absorption by the oral route varies in different animals. Mice and rabbits show signs of narcosis when given in doses of 4 ccm per kilogram of body weight. Cats and puppies behave similarly but the effects are not so marked. Dogs can tolerate an enormous dose (10 ccm per kilo. body weight) without showing any signs of intoxication. Lamson and his co-workers (1929) in studying the absorption of the drug in dogs, puppies and cats stated that after injecting doses up to 10 ccm per kilo into a loop of intestine, no tetrachlorethylene could be recovered from the expired air. Puppies however gave definite evidence of absorption; 4 ccm per kilo raised the concentration of the drug to 3 to 6.3 mgm per litre in the expired air in one and 3.2 to 4.7 mgm. in the other. Cats showed practically the same degree of absorption. Fatty substances or alcohol increase the absorption of the drug.

When dogs are anæsthetised with excessive amounts of tetrachlorethylene or when it is given intravenously, it produces a marked fall in blood pressure and temporary cessation of respiration. If the same dose is given into the portal vein instead of the femoral, there is no fall in blood pressure, possibly because the liver fixes this substance. In man the dose of tetrachlorethylene being comparatively very small, *i.e.*, 2 to 3 ccm, the effect on blood pressure is practically negligible.

Toxicity varies in different animals. Doses of 5 ccm per kilo body weight kill rabbits in 10—20 hours. Mice can stand doses of 4—5 ccm per kilo. for nearly 10 hours before death ensues. In cats, doses varying from 5—10 ccm become fatal in 6—36 hours. In dogs, death occurs with doses varying from 10—25 ccm and doses below that are generally well retained without the appearance of any toxic manifestations. The pathological changes produced in the tissues especially of the liver and kidneys which are well-marked in chloroform or carbon tetrachloride poisoning, are very slight. This will depend on the rate of absorption from the intestine. Dogs do not exhibit any signs of liver necrosis or any change in the glomeruli or tubules of the kidney. The livers of cats and puppies may show a certain amount of fatty degeneration, depending on the amount of absorption of the drug.

As an anthelmintic.

The efficacy of tetrachlorethylene as an anthelmintic, as in cases of other related compounds, depends upon the chlorine content. The efficacy is said to be increased with the amount of available chlorine. From this point of view, and also because of its being very sparingly soluble in water, the chances of absorption from the intestine are decreased. It has therefore been used in the treatment of human ankylostomiasis. The drug was found to be ineffective in human ascariasis. For trichuris infection tetrachlorethylene is considered to be superior to oil of chenopodium. A combined treatment, 1.8 ccm tetrachlorethylene and 0.6 ccm oil of chenopodium, removed 94 per cent necators and 48 per cent ankylostomes, according to some workers.

Tetrachlorethylene, used after the method described hereafter, has been stated to be at least as efficient as carbon tetrachloride, and it is safer than the latter on account of its not damaging the liver and kidneys when given in therapeutic doses. Another great advantage, especially when dealing with a large labour force, is the fact that alcohol does not increase its toxicity so much. The cure rate with tetrachlorethylene is greater than with tetrachloride, as the dose given is larger (4 ccm) than the latter.

Administration and dosage.

No very special preparation is necessary before the treatment is started. The patient should be advised to take a light diet the evening prior to treatment

and no breakfast is allowed on the day of treatment. The drug is given shaken up in two ounces of magnesium sulphate solution and the full dose for an adult is 3 to 4 ccm. When combined with oil of chenopodium, the ratio should be 3 to 1, *i.e.*, with 3 ccm of tetrachlorethylene 1 ccm of oil of chenopodium. Patients under sixteen years of age should receive a reduced dose.

The actual details of treatment recommended are as follows:—

Two ounces of saturated magnesium sulphate solution are placed in a flask or bottle of three or four ounces capacity and four ccm of tetrachlorethylene and one ccm of oil of chenopodium are added to it. The flask is corked and shaken until the drugs are distributed throughout the mixture in the finest possible droplets; the dose is then given to the patient immediately, before the drugs have time to coalesce into larger drops and float to the surface, as will happen if the mixture is left standing. This method of shaking was adopted in the first place because it was stated that it was dangerous to give tetrachloride unless it was in finely divided droplets, and the same method has been continued with tetrachlorethylene because it is considered that the even diffusion of the drug throughout the draught gives it a much better chance of coming into contact with all the worms on the gut wall and is, therefore, more efficient than if given as an undivided globule of one drachm.

Toxic symptoms.

The relative toxicity of carbon tetrachloride and tetrachlorethylene on experimental animals has been fully investigated, the use of the latter in human ankylostomiasis has been advocated because of its comparative safety. There has so far been no report of deaths with this drug and this is correlated with its low rate of absorption. Clinical observations, however, point to the fact that the drug is absorbed to some extent from human intestine, but not in amounts sufficient to produce damage of the kidney or liver cells. In one series only one case of grave intoxication in a total of 1,500 treatments was reported and in this case there were signs of giddiness which developed into unconsciousness with twitchings of the whole body. Varying symptoms of giddiness, vomiting or drowsiness in 8 out of 38 cases treated with tetrachlorethylene alone, and in 26 out of 37 cases treated with a combination of tetrachlorethylene and oil of chenopodium were reported in another series.

It seems that tetrachlorethylene would be a very safe drug to employ as an anthelmintic and the danger of toxic symptoms can be avoided if alcohol and fatty substances are withheld during treatment.

Tetrachlorethylene and chenopodium.

A combination of tetrachlorethylene and chenopodium has been used in the treatment of hook worms but seems to be little better than the use of tetrachlorethylene alone. In the case of mixed infections however viz. hookworm and ascaris, this combination is of advantage.

Tetrachlorethylene—(C_2Cl_4) is the drug of choice in hookworm infection uncomplicated with ascaris, and has entirely replaced carbon tetrachloride because of its lower toxicity. It is absorbed only in a very small degree, causing dizziness. It is contraindicated in presence of round worms and also in cases of alcoholism, gastro-enteritis, and concurrent administration of arsenicals.

The most convenient form for administration is in soft gelatine capsules each containing 0.5 ccm (8 min.). For mass treatment it may be given in a saturated solution of magnesium sulphate, thoroughly shaken up before swallowing. In children it may be given in a teaspoonful of sugar.

The adult dose is 3 to 4 ccm (45 to 60 min.), for children 0.2 ccm are given for every year of age up to 12 years of age. The following routine is prescribed.

(1) No fat or articles containing fat for 24 hours before treatment. The night before only light liquid diet; saline purgative (magnesium sulphate or sodium sulphate) between 6 and 7 P.M.

(2) Following morning give tetrachlorethylene on empty stomach with a little water. One hour after, give a brisk saline purgative.

(3) The patient should lie in bed and rest till bowels have acted freely. Allow plenty of fuit juice and sugar. No fatty food or alcoholic drink for 24 hours.

(g) Carbon tetrachloride

Carbon tetrachloride was discovered as long ago as 1849. It is chemically related to chloroform and this led to its trial as a general anæsthetic, but it was later on discarded. Hall found that it removed 100 per cent of hookworms in dogs and recommended its trial in human beings. The drug is well tolerated in such doses as 4 to 6 ccm, the only symptoms noticeable occasionally being dizziness, abdominal distress and vomiting. Further work has demonstrated beyond doubt the great efficacy of this drug in the treatment of hookworm infestations in man. The drug is toxic and has now been replaced by tetrachlorethylene.

Chemistry and properties.

Carbon tetrachloride is prepared from carbon bisulphide by direct chlorination in the presence of a catalyst such as aluminium chloride. It is also prepared by heating chloroform with iodine chloride at 165°C. when it is obtained free from contamination with sulphur compounds. It is a colourless non-inflammable liquid, having a disagreeable penetrating odour. Its specific gravity is 1.6, its molecular weight 153.8, and its melting point is—22.9°C, boiling point 77° to 78°C. It is only slightly soluble in water, freely soluble and miscible with chloroform, alcohol and other organic solvents such as petroleum, benzene, ether, fixed oils and essential oils When shaken with milk it forms a good emulsion and this, especially skimmed milk, forms a good medium for its administration.

Impurities.

Carbon tetrachloride often contains carbon bisulphide as an impurity, also traces of phosgene (carbonyl chloride) may occur. It is believed by some that these impurities, especially carbon bisulphide, greatly increase its toxicity. Strict standards of purity have, therefore, been recommended and a number of brands of the purified drug are on the market for medicinal use.

Pharmacological action.

Carbon tetrachloride is a general protoplasmic poison. *Paramoecium caudatum* and free-living *amoebae* are instantly killed in 1 in 3,000 dilution; movements of chilomastix cease in 1 in 4,000 dilution.

When applied to the skin carbon tetrachloride is a local irritant, specially when its evaporation is prevented. On mucous membranes it produces preliminary irritation followed by anæsthesia. When given by the mouth, it has a burning taste; there is a feeling of warmth in the stomach due to mild irritation of the mucous membrane. Reflex stimulation produces increased peristaltic movements of the stomach and intestine, and 1 to 2 hours after a large dose a stool may be passed. The drug passes through the stomach unchanged. That a certain amount is absorbed is evident from the fact that such symptoms as dizziness and sleepiness appear soon after ingestion. Absorption occurs through the lymphatics, especially when fatty substances are present in the gut. By this route, the drug goes straight to the systemic circulation without passing through the liver and a sufficient amount may be absorbed to cause respiratory and cardiac depression. Absorption may also take place by the portal route, and when this is the main channel of absorption, such symptoms as jaundice, vomiting, bilirubinuria usually occur, due to the toxic effects of the drug on the liver. Both clinical and experimental evidence is conclusive that if carbon tetrachloride is given with alcohol or fatty substances its toxicity is considerably enhanced. When given with olive oil, cream and absorbable fats, animals quickly show signs of intoxication. Patients feel drowsy and have a desire to go to bed. These observations show the importance of controlling the diet when the drug is given. Fats should be excluded from the diet. With non-absorbable fats such as castor oil or liquid paraffin, the absorption is not so much increased, but it is preferable to discard fatty substances altogether. Large doses do not appear to be proportionately more harmful than small ones; in other words, the

drug appears to have a tendency to protect against itself. Even small amounts such as 0.1 ccm dissolved in alcohol and given intravenously have a marked toxic effect on the heart and respiration, whereas after oral administration and injection into the mesenteric veins, these effects are negligible on account of the detoxicating action of the liver. In patients with cirrhosis where the liver parenchyma is damaged, severe toxic effects on the heart and respiratory system have been recorded.

Action on Liver.

If an injection of a fairly big dose of carbon tetrachloride is given into the mesenteric vein of an animal, there is acute destruction of the liver cells, and jaundice develops. When absorbed from the gut,, the drug is taken to the liver and produces fairly generalised fatty degeneration; with larger doses, necrosis develops, usually beginning in the centre of the lobule and spreading to the periphery. The liver function test (phenol-red test) may show great derangement of function. In severe cases the hepatic cells at the periphery of the acini show widespread infiltration with droplets of fat. In cats after such small doses as 0.25 ccm per kilo the liver tissue is practically all destroyed. This liver damage reaches its maximum in 48 hours, after which rapid repair sets in, and in a few days little evidence of damage is left. In therapeutic doses in man some injury to the liver in the form of fatty infiltration probably always occurs, and perhaps some slight fatty degeneration and central necrosis. But where the liver is originally healthy and due precautions are taken to avoid excessive absorption, this is not sufficient to disturb the normal functions of the liver nor to cause any permanent injury. Any damage to the liver which may result is rapidly made good if the drug is not repeatedly administered. Continued and prolonged administration of carbon tetrachloride in dogs produces cirrhosis of the liver, but no damaging effect on the pancreas.

Heart.

Carbon tetrachloride has a depressing action on the heart and respiration. It slows the beats of the heart, the contractions become weak and the blood pressure falls. The amount of bilirubin is increased and calcium is decreased in the blood. After severe injury to the liver, guanidine and guanidine-like substances in the blood are increased and there may be hypoglycæmia. Administration of calcium salts is beneficial in this condition. It has been shown that acute intoxication is more likely to occur when the dietary calcium intake is reduced. The blood in these cases shows decrease of calcium content and there are convulsive symptoms resembling tetany. The administration of calcium chloride intravenously or calcium and parathyroid extract orally produces a rapid cure in experimental animals. It has also been shown that after severe injury to the liver there is definite retention of guanidine in the blood, bilirubinæmia and hypoglycæmia. In dogs symptoms of nervous irritability, gastro-intestinal irritation and hypoglycæmia can be produced by giving guanidine hydrochloride. When calcium is given to these animals the symptoms abate. The administration of ammonium chloride and hydrochloric acid also counteracts the toxic effects of carbon tetrachloride by increasing the ionised calcium of the blood.

Nervous system.

The action of carbon tetrachloride on the nervous system resembles that of chloroform. Slight giddiness and a feeling of relaxation and drowsiness are the only symptoms noticed after therapeutic doses.

Kidneys.

Next to the liver, the kidneys are the organs most severely affected in experimental animals. In dogs large doses produce cloudy swelling of the convoluted tubules and fatty infiltration. In cats the effects are much more severe. It is probable that the drug only reaches the kidneys in sufficiently high concentration to injure them if the liver is damaged and cannot adequately deal with it. Carbon tetrachloride is well tolerated by pregnant women. The drug relaxes the isolated uterus.

Excretion.

Some of the drug is excreted in fæces unchanged; some is absorbed and is excreted through the lungs, in fact, these organs are the main channels of excretion whatever may be the mode of administration. Only traces are found in the urine. According to Robbins (1929) the drug is not excreted by the kidneys.

Dosage and administration.

Dosage and method of administration. The dosage and method of administration have varied greatly in different hands. Though doses ranging from 1.5 ccm to 10 ccm have been tried by eminent workers, 3 ccm may be considered to be a safe dose for an average adult. Chopra and McVail (1923) employed a 60 min dose of the chemically pure drug with excellent results and with no untoward effects. A single dose of 3 to 4 ccm is safer than a smaller quantity given in divided doses, since by the latter method more of the drug is absorbed into the circulation. The anæsthetic protective action of the drug itself on the mucous membrane of the intestine is probably an important factor in limiting absorption.

The drug has been administered in various ways. Hall recommended that it should be administered in hard gelatine capsules. The capsule is usually of 1.0 ccm capacity and care should be taken before it is put in the mouth that it is covered tightly. It should be swallowed promptly to avoid the chance of inhaling the drug into the larynx and trachea, which may produce collapse or inhalation pneumonia. A simple method is to administer the drug in water with or without a subsequent dose of magnesium sulphate. The drug is poured in a tablespoon or a small glass and covered with water. As it is insoluble in water and very heavy it remains at the bottom and the patient gets water first, then the drug and then more water. Magnesium sulphate has also been used as a vehicle with success. The action of the drug is not impaired by simultaneous administration of salts though it is said by some to increase its irritant action on the intestinal mucosa. The saline purgative can be given either with the drug or one to two hours later. Simultaneous administration of a saline purgative does not reduce the anthelmintic activity of the drug as is believed to be the case with chenopodium. Chopra and McVail used skimmed milk as a vehicle. The drug forms a good emulsion with it, the disagreeable taste is to a large extent concealed and the toxicity is not increased. Given by this method carbon tetrachloride produces a high percentage of cures with one treatment, probably on account of more thorough distribution of the emulsified drug in the intestines.

There is no necessity for preliminary fasting with this drug. The patient takes his usual evening meal and takes carbon tetrachloride the next morning, no food being taken for three hours after. Administration of the drug, immediately before or after food is not advisable. No fat or alcohol should be allowed a few days before or after the treatment.

Anthelmintic effects.

There is no doubt that carbon tetrachloride is the most effective drug known for the expulsion of hookworms of the genus *Necator*. In doses of 3 to 4 ccm one treatment removes 95 to 98 per cent of the worms and completely cures 60 to 70 per cent of cases of pure necator infections. It is much less effective against ankylostomes and ordinarily does not completely cure more than 30 to 40 per cent of cases though probably 80 to 85 per cent of the parasites are expelled. The drug has little effect on ascaris. On enterobius carbon tetrachloride has a very decided action and large numbers of parasites are removed by a single dose of 3 to 4 ccm by the mouth. Its effectiveness cannot, however, be accurately measured as reinfections very commonly occur. Enterobius infections can also be treated by enemata of 6 oz of warm skimmed milk to which 6.0 ccm of carbon tetrachloride are added. The patient is generally given a strong dose of salts the previous night to empty the lower bowel and before the enema is given the foot end of the bed is raised and the patient lies on the left side. The parasites expelled are all dead. Trichuris and strongyloides are not affected. Tænia and hymenolepis are not destroyed in the dog but in man the drug is of great value in removing both *T. solium* and *T. saginata*. Cure rates of 84.6 per cent were obtained in a series of thirteen cases. The patients, on the day of admission, were given a meal of milk and bread only and first thing the next morning they were given a dose of carbon tetrachloride, shaken up in a saline purge (saturated magnesium sulphate solution). Carbon tetrachloride would, therefore, appear to be a better drug than male fern for the removal of the tapeworms, though male fern has held the field for such a long time. Carbon tetrachloride is also effective in the treatment of fasciolopsiasis and has been used in combination with tartar emetic in the treatment of schistosomiasis.

Advantages.

Advantages of carbon tetrachloride over other anthelmintics except tetrachlorethylene are:—

(*a*) A high degree of efficacy against hookworms.

(*b*) No preliminary dietetic treatment is necessary except that fats and alcohol should be avoided a few days before and a day or two after the treatment.

(*c*) The patient need not remain in bed and thus need not be away from his usual work.

(*d*) The entire treatment can be given at one time.

(*e*) The cost is nominal, a dose of carbon tetrachloride costing only a few pice.

The last three points are specially important when dealing with large labour forces.

Toxic effects.

The only symptoms complained of in human beings after therapeutic doses of carbon tetrachloride are a sensation of warmth in the stomach, slight nausea, giddiness, confusion of thought and a feeling of mild alcoholic intoxication an hour or two after its administration. The patient has a taste of boiled cabbage in the mouth after eructations. Vertigo, cephalalgia and somnolence of a transitory nature may occur. A feeling of relaxation and drowsiness follows, and the patient may fall asleep. Rarely, there is an uncomfortable

feeling in the epigastrium, followed by diarrhœa. When excessive absorption occurs or larger doses are given there is stupor, dilatation of the pupils and irregularity of pulse; jaundice may be produced and rarely pain in the lumbar region, hæmaturia and albuminuria. Death may occur from collapse and extensive lesions in the liver and the kidneys have been seen. Sometimes deaths have occurred with small doses such as 45 minims (3.0 ccm). In a million and a half cases treated in Fiji, there was one death in 50,000. All deaths occurred in alcoholics or persons heavily infested with ascaris, where mechanical obstruction leads to increased absorption. This danger can be overcome by prescribing the drug with chenopodium. The habitual use of alcohol and a damaged liver increase the susceptibility to toxic effects.

If symptoms of poisoning occur a brisk purgative should be administered. If liver damage has occurred only symptomatic treatment is indicated. If muscular irritability is present calcium might be useful in view of the fact that there is marked calcium deficiency in the blood following bilirubinæmia. Beathie et al have successfully treated a case of acute carbon tetrachloride poisoning by the administration of methionine. It is thought that the cause of liver disturbance induced by carbon tetrachloride is abnormal metabolism of methionine and related compounds.

Methionine.

There is a good deal of controversy regarding the toxic effects produced by carbon tetrachloride. Some workers pronounce it as being very poisonous and are of opinion that it should not be used under any condition. Others consider it to be a harmless drug. Clayton Lane (1930) cited fatalities with such doses as 1.5 ccm and considers that it is a retrogressive measure to use carbon tetrachloride in preference to thymol. He does not consider the drug safe for mass treatment. The author's experience is that if the cases are selected properly and necessary precautions are taken, carbon tetrachloride is a safe drug. Thymol is not only more expensive but is liable to produce toxic symptoms more frequently. Deaths have also been recorded after thymol administration for helminthiasis.

Precautions and contra-indications.

The drug should be pure and the dose should not exceed 3.0 ccm. The whole dose should be given at one time and not divided. It should always be accompanied or followed by a saline purgative. The drug is contra-indicated in badly nourished and weak individuals and those having a low calcium content in the blood. If anæmia is present give iron. Under no circumstances should it be given when cirrhosis or other organic disease of the liver co-exists or when there is advanced kidney disease. A diet rich in carbohydrates, proteins and calcium is beneficial. It is better to administer the drug three hours after a moderate meal. Some recommend milk or calcium lactate for several days before taking carbon tetrachloride. Preliminary starvation is dangerous. A treatment with carbon tetrachloride should not be given for several days after chloroform anæthesia and it should be given to alcoholic subjects with caution. Idiosyncracy to carbon tetrachloride may exist and small doses may produce very marked toxic symptoms.

Carbon tetrachloride can be given to malaria and kala-azar patients, preferably during the afebrile period. It is well tolerated by pregnant women and children. When heavy ascaris infections are present, it should not be given alone but in combination with oil of chenopodium.

Carbon tetrachloride and chenopodium.

A combination of carbon terachloride and oil of chenopodium is more effective than either of the drugs alone. The reason is not far to seek. Carbon tetrachloride itself is effective against pure necator infections and chenopodium alone for ascaris infections, whereas ankylostoma infections are readily cured by a combination of the two with a relatively high proportion of chenopodium. The effects of the two drugs on the host are independent. One does not increase the toxicity of the other while their action is supplementary to each other as far as the parasites are concerned. Dilution of chenopodium with carbon tetrachloride lessens the discomfort of taking the former drug and reduces the cost of treatment. The dose of the two drugs is so reduced as to be below the toxic limit.

Different proportions of these two drugs have been combined by various workers. Smillie and Pessoa use 2 ccm of carbon tetrachloride with 1.ccm of oil of chenopodium or 0.5 ccm of ascaridol. Soper (1924) pointed out that the proportion of the two drugs should depend on the nature of the worms harboured. Chopra and Chandler (1928) recommend for predominantly ankylostoma infections, or where ascaris is abundant, 2 ccm of carbon tetrachloride and 20 min. of oil of chenopodium; when ankylostomes are less numerous than necators 3 ccm of carbon tetrachloride with 10 to 20 min of chenopodium are preferable. A mixture of carbon tetrachloride and oil of chenopodium in proportion of 3 parts to one part is considered a safe and efficient anthelmintic against. *T. saginata.* Daubney and

Carman, in a series of thirty patients had a cure rate of 97 per cent in what they described as light infections. Maplestone and Mukerji, however, consider that carbon tetrachloride, by itself, given in a single dose, is as effective as the mixture.

Oil of chenopodium and carbon tetrachloride are readily miscible but the latter drug is more volatile and if the mixture is old the proportions will change. It is advisable to prepare it fresh. The disadvantage of this combination is that it is extremely obnoxious to take. Chenopodium can be given separately in a capsule, followed by carbon tetrachloride in water, salt or milk, either immediately or one or two hours after. When mass treatment is given the mixture is more convenient.

PREPARATIONS.

Preparations

Carbon tetrachloride (U.S.P.X.) Synonym.—Tetrachlorethane. Commercial name—Necatorine. Dose 60 min. (3.0 ccm) as anthelmintic for adults.

Children of one year should be given 10 min., for a child of 10 years 30 min., and a youth of 16 years 40 min. Milk is the best vehicle for administration. Capsules of carbon tetrachloride are available containing 20 min. in each (P. D. & Co.); capsules of 0.3 ccm, 1 ccm and 3 ccm are also marketed.

(c) Other Carbon Derivatives

It has already been pointed out that compounds of chlorine with ethane, ethylene, and methane series show anthelmintic properties and even compounds of the propane and butane series have been tried. Ethylene dichloride or dichlor-ethylene, $C_2H_4Cl_2$ is inferior to C_2Cl_4 which is as effective as CCl_4. Carbon trichloride or hexachlorethane, C_2Cl_6, is entirely ineffective; chloroform $CHCl_3$ has only slight anthelmintic action. It would appear that high solubility is correlated with increased absorption and consequent lowered anthelmintic action and increased toxicity. Very low solubility has the opposite effect and as the solubility becomes less and less the anthelmintic activity decreases. From our experience with these compounds it appears that solubility of something like that of carbon tetrachloride (1 in 1,250 of water) is the best from an anthelmintic point of view.

Chloroform.

Choroform.—Chloroform is a well-known general anæsthetic, but it has been used very rarely internally except in small doses. The dose in the British Pharmacopoeia is 1 to 5 minims, but as an anthelmintic as much as ½ to 1 drachm (2 to 4 ccm) has been given. When administered in small doses and in a diluted form, its irritant action on the gastro-intestinal tract is not marked, the carminative and sedative action being more prominent.

Anthelmintic effects.

As an anthelmintic, chloroform has been used either alone or in combination with other substances. It was used against tapeworm infections and was tried in hookworm infections of dogs with good results; a mixture of chloroform and castor oil for use in man, did not prove to be effective though many worms were expelled. The results on ascaris and whipworms were also not very striking. Chloroform in combination with oil of chenopodium is more potent than choloroform alone and has been shown by Caius and Mhaskar (1923) to expel a high percentage of both ascaris and hookworms. A vigorous after-purgative is always necessary after its administration as most of the worms do not die but get benumbed and hence should not be allowed to recover from the stupefying effects of the drug. Chloroform used for internal administration must be of the purest quality, freed from all the common impurities such as hydrocarbons, chlorides, chlorine, free acids, phosgene, etc. The drug is no longer used as an anthelmintic since the introduction of more effective and comparatively less toxic carbon tetrachloride.

Toxic symptoms.

These resemble the symptoms seen after carbon tetrachloride. Even 30 to 45 min produce unpleasant symptoms and as the dose is increased beyond 45 min the toxic effects become more marked. Dogs, after two doses, of 3 ccm each, refuse food, vomit, are seized with mania-like symptoms, and die. Extensive changes in the liver are noticed in these animals, the lobules becoming yellow in colour and there is marked degeneration of the parenchyma, which shows mottling and there are hæmorrhages in lungs, stomach, intestines and diaphragm. In man the symptoms produced are those of gastro-intestinal irritation, but after absorption of large doses, drowsiness, loss of consciousness, coma, and death may occur. Fatty degeneration and central necrosis of the lobules of the liver occur, as in the case of carbon tetrachloride. Collapse and death may occur from severe gastro-intestinal irritation.

Eucalyptus oil and chloroform mixture.

A mixture of eucalyptus oil and chloroform was introduced under the name of Manson's mixture. It contains eucalyptus 2 gm, chloroform 2 gm., and castor oil 40 gm. Most of the authorities who have tried this mixture agree that it is dangerous and not efficacious. Exclusion of eucalyptus from the mixture does not lower its anthelmintic efficacy showing that the main action is due to chloroform.

Iodoform.

Iodoform has occasionally been used for removing ascaris in man, in doses of 0.01 to 0.06 gm three times a day in combination with sodium carbonate. Hall and Foster found this drug useless as an anthelmintic in dogs in much larger doses than those used in man.

Phenothiazine.—This dye has been recommended by Manson Bahr (1940) for removal of ascaris in doses of 8 gm daily for 4 days in adults. An after purge of sodium sulphate may be used if necessary.

(3) The Essential Oil Group

(a) Oil of Chenopodium

SYNONYMS: AMERICAN WORM SEED, MEXICAN TEA

Chenopodium has been used as a household remedy against intestinal parasites for a long time. It was used by American Indians in the days of Columbus. The oil was originally used many years ago against ascaris, but was not popular because of the toxic and sometimes fatal effects produced in many cases. Schuffner and Verwoort (1913) tried it against hookworm in Sumatra in doses of 3 ccm in combination with castor oil and chloroform with excellent results. From that time the drug came rapidly into use. It came to the forefront during the First Great War when the supply of anthelmintic drugs such as santonin and thymol was curtailed. On account of its toxicity it is not much used now.

The active oil is obtained from *Chenopodium ambrosioides* var. *anthelminticum,* or the *American wormseed* belonging to the N. O. Chenopodiaceæ or the goosefoot family. A number of other species also yield the essential oil and *Herba chenopodia* is official in the Austrian Pharmacopœia. In India at least seven species of chenopodium grow, but only two of them yield oil having anthelmintic properties, these being *C. ambrosioides* and *C. botrys.* A variety of *C. ambrosioides* is cultivated in Java.

Chemical composition and properties.

Chenopodium contain 40 to 80 per cent of *ascaridole* which is chemically allied to essential oils; this is the anthelmintic as well as the toxic constituent. The oil has no definite boiling point and when it is heated to 100°C. in the air it explodes with violence. Different specimens differ much in their physical characters, the colour may vary from pale yellow to light golden yellow. The toxicity of different stocks also varies greatly and the samples met with in the market are often adulterated up to the extent of 50 per cent. The whole oil as well as ascaridole are practically insoluble in water and are very slightly absorbed; on this quality its anthelmintic action is chiefly dependent. Ascaridole is altered by heat into a less volatile substance which has much weaker anthelmintic properties. Distillation should therefore be carried out with great care.

Besides the volatile substances chenopodium also contains certain terpenes—limonene, cymene and terpinene. Specimens of chenopodium oil met with in commerce vary a great deal in activity on account of variations in their ascaridole content. The drug now on the market is properly standardised.

Pharmacological action.

Oil of chenopodium like other volatile oils is diffusible and irritant to the skin and mucous membrane. It has germicidal properties. It kills protozoa and was at one time recommended in amœbic dysentery. Internally the oil has a sharp burning taste, produces salivation and reflexly stimulates the flow of gastric juice. The peristaltic movements of the intestines are decreased and a constipating effect is usually produced. Large doses produce severe irritation of the whole of the gastro-intestinal tract. The oil is absorbed from the stomach with difficulty, but absorption may take place from the intestines.

The oil has a markedly depressant action on the circulation, the blood pressure shows a considerable fall in experimental animals after intravenous injections of 0.02 ccm per kilo. The pulse becomes slow and weak; there is also marked depression of respiration.

The oil has a well marked effect on the central nervous system. There is at first a

transitory stimulation followed by a marked depression of the respiratory, cardio-vascular and other centres in the medulla. Later, the higher centres are affected, giving rise to stupor and coma. The special senses, especially sight, may be affected. Cases of blindness and deafness have been reported after use of chenopodium oil.

Whether given by the mouth or intravenously the oil is partly excreted by the lungs and partly by the kidneys. The elimination is slow and neither the oil nor the products of its decomposition can be readily detected. Small doses have a diuretic effect by mildly irritating the kidneys; large doses may set up inflammation and albuminuria. Subcutaneous injections of the oil in animals set up renal irritation.

In vitro experiments show that the action of the oil is probably due to penetration through the cuticle and into the cells of the parasites, resulting first in stimulation and then paralysis of the musculature. In such dilutions as 1 in 5,000 to 1 in 10,000 there is at first tonic contraction of the muscles of ascaris, followed by relaxation and paralysis, so that the parasites cannot resist the intestinal peristalsis. Experiments on earthworms show that the toxic effect is much greater at 37°C than at 21°C.

Anthelmintic effect.

Oil of chenopodium has a marked action on all kinds of intestinal nematodes, but little or no action on large tapeworms. It is particularly effective against ascaris and is as good an ascaricide as santonin, a very high percentage of worms being removed even if a complete cure is not effected. The drug does not kill the parasites but only paralyses them, and they must be expelled by free purgation. Against hookworm infection chenopodium is a particularly useful drug. It is less effective against necators than carbon tetrachloride, but is about equally effective against ankylostomes. In doses of 30 min it can be depended upon to remove about 90 to 95 per cent of necators and about 80 to 85 per cent of ankylostomes.

The oil is not so effective against trichuris because of the situation of these worms in the colon and cæcum. Large numbers of threadworms are expelled but here also the effectiveness is less than on the worms situated higher up in the gut. Carbon tetrachloride is probably more effective against these worms. Its anthelmintic efficacy against clonorchis, hymenolepis and strongyloides is doubtful. Intramuscular injections of chenopodium or ascaridole are said to be useful in filariasis, the initial dose being 0.5 ccm gradually increased to 2.0 ccm. Injections are said to cause disappearance of filaria in many cases.

Dosage and method of administration.

The maximum dose of the oil is 3 ccm given in three doses of 1 ccm each, at intervals of an hour. The last dose is followed one hour later by an ounce of saturated solution of magnesium sulphate. This dosage cannot be adopted in general treatment, because it frequently gives rise to severe toxæmia especially in ill-nourished patients.

Most of the authorities recommend 1.5 to 2.0 ccm (30 min.), divided into two or three doses, given at intervals of one hour. This method is both safe and efficient. Doses recommended for children are, 3 capsules of 2 min. each for a child of 3, 3 min each for a child of 5, 5 min each for a child of 10 and 7 min each for a child of over 12 years. Weak individuals should have reduced doses, according to their state of health.

The drug may be given in the morning in doses of 10 to 15 min. at 7, 8 and 9 a.m. Some authorities allow an interval of 2 to 3 hours between the doses and some prefer to give the anthelmintic and purgative together, 10 to 20 drops being given with senna or mixed with castor oil. To conceal the taste it may be mixed with olive oil or almond oil.

Pure ascaridole is now on the market and is used in doses of 1.0 ccm for an adult given in 2 or 3 divided doses an hour apart. Pessoa (1923) gave 1.0 ccm in one dose in hard gelatine capsules on an empty stomach, followed by a saline purgative half an hour later; a preliminary purgative is not necessary. For children up to 5 years, 1 to 2 drops of ascaridole in an emulsion of 10 to 20 ccm of castor oil is the best. For children between 6 and 15 years, one drop for each year of age in gelatine capsules followed an hour later by a saline purgative is generally recommended.

Chenopodium has a very obnoxious and nauseating taste and is preferably given in hard gelatine capsules, although it can be taken in syrup or in a lump of sugar. The results, obtained when given in the form of emulsion, are inferior to those when the oil is given by itself. It has also been shown that the drug is less toxic and its anthelmintic effects are not only as good but better if no preliminary dietary measures are adopted and no pre-purgative is given. This makes the oil a useful drug for labour forces in the field. It is preferable to give the oil three hours after a moderately light morning meal.

Necessity of a purgative.

A post-purgative should always be administered after chenopodium. It has been pointed out that chenopodium inhibits intestinal peristalsis and thereby tends to produce constipation. The administration of castor oil immediately after it retards the absorption of the drugs and helps its elimination from the alimentary canal. Certain fixed oils such as olive oil prevent the toxic effects of chenopodium. If a saline purgative is to be used it should be given at least 2 hours after administration of the oil as it has been shown that simultaneous administration of salts greatly reduces the anthelmintic efficacy of the drug.

Chenopodium has been tried intramuscularly and is said to have an anthelmintic effect when given in this way. The mixture prescribed for this purpose consists of chenopodium oil 60 ccm, camphorated oil 60 ccm and resorcin 4 gm. The usual dose is 4 ccm of the mixture injected into the buttock. Its vermicidal action against hookworm is small but is quite efficacious against trichuris. Intravenous injections of the oil in doses of 2.0 ccm have been given, with the idea that after absorption into the blood the oil will reach the hookworms, which have anchored themselves to the mucous membrane and which will then let go their hold. Given in this way the oil may produce a mild collapse.

Toxic effects.

The oil of chenopodium is a toxic substance and causes severe irritation of the mucous membranes. Rabbits die of vomiting, convulsions, coma and paralysis after 0.5 ccm doses; 0.25 ccm per kilo is fatal in cats. Fish die in dilution of 1 in 8,000 in water and are narcotised in 1 in 25,000. A full dose in an animal if repeated within a few days may cause death, and sensitiveness to the oil persists for at least 5 to 9 days. This should be remembered when treating unsuccessful cases in man. In man it sets up severe gastro-intestinal irritation; such a dose as 3 ccm produces nausea, vomiting, severe abdominal pain and finally paralysis of the gut. A certain amount of dizziness is nearly always present and there may be slight deafness and ringing in the ears. After excessive doses extreme dizziness, a feeling of intoxication, prostration, headache, drowsiness and unconsciousness occur, showing that there is severe toxæmia. The face is flushed, respiration slow, pupillary reaction sluggish and convulsions may follow giving place to flaccid paralysis. Albuminuria is present in severe cases and there may be hæmaturia. Autopsy shows severe inflammation of the gastro-intestinal tract, liver, and kidneys, and hyperæmia of the brain; there are fatty changes in the parenchyma of the liver and the kidneys. Deafness may sometimes persist. Children are more susceptible to the effects of the oil than adults and it is advisable not to exceed the prescribed dosage. It is better to use some other anthelmintic in children under 5 or 6 years of age and in very old and debilitated individuals. Owing to its slowness of absorption the toxic symptoms may appear a day or so after administration, and cumulative effects may be produced by smaller doses several days apart. In man death has been reported from ingestion of half an ounce or even less. Tolerance is decreased by starvation and debility, and increased by fixed oils.

Treatment poisoning.

As soon as inordinate sleepiness and depression or any other symptoms of intoxication appear, the administration of the drug, if all the capsules have not been given, should be stopped and a brisk purgative given. The stomach should be quickly washed out. There is no antidote known and the treatment is, therefore, entirely symptomatic. Respiratory and cardiac stimulants should be given.

Precautions and contra-indications.

Chenopodium oil should be given with caution and in small doses when disorders of the heart and kidneys are present. It is forbidden in chronic nephritis and organic disease of the heart, and where hepatic and gastro-intestinal disorders are present. Prior to the administration of chenopodium against hookworm it is better to administer iron and arsenic and liver extract, especially when the patients are anæmic.

Preparations.

PREPARATIONS.

Oleum chenopodii (U.S.P.X.). It is distilled with steam from the fresh above-ground parts of the flowering and fruiting plant of *Chenopodium ambrosioides*. Dose 10—30 min. (1 to 2 ccm); for children as many minims as the number of years up to 10 years.

Ascaridole (N. O.). Dose 1.0 ccm. A synthetic ascaridole has been prepared which in doses of 4 drops for each year of age of children and 70 drops for an adult is an effective anthelmintic.

(b) Other Essential Oils Used as Anthelmintics

A number of essential oils have been used in the treatment of intestinal worms but none of them have any specific action against any of the parasites.

Oleum eucalypti. The oil is distilled from the leaves of *E. globulus, E. dumosa* and a number of other species. The chief constituents of the oil are pinene, eucalyptol (cineol) and aldehydes of fatty acids. It has been used as an anthelmintic in combination with chloroform but its action appears to be very mild. Caius and Mhaskar (1920) gave it in 40 min. doses in the form of a mixture with gum acacia and castor oil. Doses of 60 min. and over produced giddiness, and vomiting, probably owing to the presence of aldehydes. It was quite ineffective against hookworms and had little or no effect against ascaris and whipworms. Pure encalyptol has also been tried; it is much less toxic but the vermifugal action against intestinal parasites is feeble. *Eucalyptus oil.*

Oleum cajuputi. Cajuput oil is a volatile oil distilled from the fresh leaves and twigs of *Melaleuca leucadendron,* var. *minor, M. viridiflora.* It is a green-coloured oil with an aromatic odour and a pungent taste. The chief constituents are: cajuputol, which is the same as eucalyptol or cineol, terpineol, small amounts of pinene and traces of aldehydes. The oil has, therefore, the same active principles and same action as oil of eucalyptus. *Cajuput oil.*

It has been used as an anthelmintic in the gold mines of Guiana in the treatment of mild hookworm infections. Caius and Mhaskar (1920) tried the oil and found that even in 60 min. doses (the maximum tolerated dose) hookworms were very feebly affected and ascaris and whipworms were not attacked at all. The oil of cajuput cannot, therefore, be recomended as an anthelmintic.

Oleum terebinthinae. Common turpentine is an oleoresin obtained from several varieties of pines, chiefly from *Pinus palustris.* There are several varieties of turpentine oil on the market but the rectified oil is the only variety used for pharmaceutical purposes. Pure oil is a colourless liquid chiefly composed of one or more terpenes. Its anthelmintic dose is ½ to 4 drachms usually combined with castor oil. *Haustus terebeni* is sometimes prescribed; it consists of the oil combined with a drachm of glycerine and an ounce of cinamon water. *Oil turpentine.*

Oil of turpentine is irritant to the skin and mucous membrane. It is readily absorbed from the alimentary canal and circulates in the blood unchanged. It is excreted through the skin and lungs but mainly through the kidneys, partly unchanged and partly as terpenols in combination with glycuronic acid.

Oil of turpentine is a toxic drug and half an ounce has proved fatal in children. In larger doses in adults severe gastro-intestinal irritation is set up, followed later by acute nephritis and painful micturition. The medullary centres are stimulated, producing at first excitement, delirium, and convulsions and later paralysis. Pain in the chest due to bronchitis is set up by irritation of the respiratory passages. If symptoms of poisoning occur the gut should be evacuated by large doses of magnesium sulphate; stimulants such as coffee should be administered.

As an anthelmintic, the oil of turpentine in doses of ½ to 1 ounce has been successfully used in man. Some authorities have discouraged its use on the ground that it is likely to produce nephritis during excretion. In large doses, as used for expelling worms, it acts as a purgative and is not absorbed in sufficient quantities to cause renal irritation. Tapeworm infestations have been treated with one ounce doses in combination with castor oil with satisfactory results. Caius and Mhaskar (1920) treated a series of cases of hookworm with oil of turpentine, but the results were disappointing. All the evidence is, therefore, against its use as an anthelmintic except possibly for tapeworms. Saline or soap enemata containing an ounce of turpentine are effective in expelling oxyuris.

Oleum tanceti (Tansy.) This oil is distilled from the flowering tops and leaves of tansy, *Tanacetum vulgare* belonging to N. O. Compositæ. It is a yellowish brown liquid consisting of thujone, borneol, camphor and a number of resins. It has been tried as an anthelmintic but is found to be wholly ineffective against hookworm, ascaris and whipworms. It is also a toxic drug and even in therapeutic doses produces vomiting and giddiness. The leaves and tops of *Tanacetum vulgare* have been used in the form of an infusion or as an enema to expel ascaris and oxyuris. *Oil of Tansy.*

Oleum absinthi. The oil of absinth or vermouth or wormwood is obtained from the flowering tops and leaves of *Artemisia absinthium* belonging to N. O. Compositæ. It is usually a dark green oil consisting of a number of volatile principles and a bitter substance called *absinthin.* Medicinally absinth was chiefly used as a febrifuge and as an anthelmintic. It is a fairly toxic drug and is seldom used at present. The oil was tried by Caius and Mhaskar (1920) in the form of an emulsion against hookworms but the results obtained were not encouraging. *Oil of absinthi.*

Oil of cloves.

Oleum caryophylli. The oil of cloves is a thick yellow oil distilled from the flower buds of *Caryophyllus aromaticus.* The chief constituents of this oil are an unsaturated phenol called *eugenol* which forms 80 to 90 per cent. of the oil, acetyl derivative of eugenol (2 to 3 per cent.), caryophyllene, etc.

The oil has been tried against hookworms with little success. Caius and Mhaskar (1922) by using an oil containing 92 per cent. eugenol in the form of an emulsion obtained satisfactory results. With 90 min. doses, administered in divided doses at half an hour intervals, 64.3 per cent of hookworms were expelled, necators being much more vulnerable than ankylostomes. Oxyuris are also expelled in large numbers; ascaris and trichuris are not affected.

Pure eugenol and iso-eugenol have also been tried and found to have well marked vermicidal action against hookworms, their action resembling thymol. Both are well tolerated in doses of 1 to 1½ drachms, and with such dosage they produce a mild purgative effect, thus obviating the necessity for an after purgative.

Betel oil.

Oleum betulae. Betel oil is an essential oil obtained from the Indian betel vine commonly known as 'pan'. Leaves of this plant contain 39 to 70 per cent of phenols consisting mainly of eugenol mixed with a small percentage of betel phenols. The oil in doses ranging from 30 to 90 min. has only a weak anthelmintic action against hookworms, expelling less than 20 per cent of these parasites. The other intestinal parasites are not touched. The popular belief regarding the anthelmintic effect of betel chewing can be accounted for by the elimination of immature hookworms from the body by constant spitting and so preventing them from migrating from the trachea into the œsophagus.

Sassafras oil

Oleum sassafras. The oil of sassafras is an essential oil distilled from the root of *Sassafras venifolium.* It contains 85 per cent. of safrol and has powerful antiseptic properties resembling those of clove oil. According to Caius and Mhaskar a dose of 30 to 60 min expels 52.4 per cent hookworms; other intestinal parasites are not affected. The oil resembles chenopodium oil in its action, but it is much less toxic to the host. Pure safrol has also been used in 60 min. doses and has been found a fairly good anthelmintic against hookworms.

Oil anisi.

Oleum anisi. This is obtained from distillation of ripe fruit of *Pimpinella anisum.* The oil is mainly composed of anethol but also contains small quantities of terpene. Its action closely resembles that of thymol. It is a fairly good vermicide, the maximum dose of 60 min. expelling about 50 per cent of hookworms.

Pure anethol has also been used and is quite effective against hookworms expelling as many as 70 per cent of the parasites. No toxic symptoms are met with either with the oil or with the active constituent anethol, even in one drachm doses.

Oil of cinnamon.

Oleum cinnamomi. The oil is derived from *C. zeylanicum* and it contains large quantities of cinnamic aldehyde (50 per cent) and is a powerful germicide. The oil has been tried but with disappointing results against ascaris, trichuris or hookworms.

Rue oil.

Oleum rutae. The oil is derived from the fresh leaves of common Rue, *Ruta graveolens.* It was used as an anthelmintic in India and is still used in some parts, the juice of the plant being given to children to expel intestinal parasites. Its anthelmintic efficacy is, however, slight.

Oil of copaiba.

Oleum copaiba. It is quite useless as an anthelmintic even in doses of 30 to 60 min. against ankylostomes, necators, ascaris and trichuris.

(4) Santonin

Sources of santonin.

Santonin is a classical remedy for the treatment of ascaris and oxyuris infections. It is obtained from *Artemisia maritima,* a plant whose flowering tops were used by the ancient Greek and Roman physicians as a remedy to expel intestinal parasites. Santonin is one of the most expensive drugs. Before the War practically the whole of santonin supply of the world came from Russian *Artemisia cina.*

A number of allied species such as *A. maritima*, var. *stechmanniana* and *A. pauciflora* (*A. anthelmintica*) also grow in Turkestan. Owing to political and economic disturbances in Russia some years ago there was great scarcity of santonin, and *A. cina* having good santonin content was successfully cultivated in Holland. *A. maritima* or *A. brevifolia* grows

abundantly in a state of nature in the dry inner valleys of the western Himalayas from Kumaon to Kashmir at an altitude of 4,000 to 12,000 feet above the sea level, and recently santonin has been obtained on a commercial scale from this source. Large quantities of artemisia with high santonin content are found to grow wild in the Kurram valley in the North-Western Frontier not far from the railway line.

Chemical composition and properties.

The active principles of *A. maritima* are:—(1) A neutral principle, santonin which is responsible for the anthelmintic properties, (2) a bitter principle, artemisin (oxysantonin) which is not very active, (3) a volatile oil, and (4) santonic acid. The amount of santonin extracted from the Russian Artemisia is usually 1.2 to 1.4 per cent, but may be as high as 2.3 to 3.6 per cent. The yield from the artemesia growing in Kashmir does not exceed 1.0 to 1.5 per cent and the yield from that of Kurram Valley is slightly less. Santonin is a crystalline anhydride or lactone of santonic acid; it occurs in colourless, shining, flattened, rhombic prisms or as white crystalline powder. It is stable in the air, but on exposure to light develops a yellow colour due to the formation of an isomeride of santonin called chromosantonin.

Pharmacological action.

Externally santonin has very little action. Taken by the mouth, it is tasteless at first but develops a bitter taste after a short time. Santonin, unlike most other anthelmintics is not a gastro-intestinal irritant. It is quickly absorbed from the empty stomach. In the small intestine, the drug is acted on by the alkali present, sodium santoninate being formed. The sodium salt is soluble and is absorbed, but its effect on the circulatory and respiratory systems is slight in therapeutic doses. With large doses the symptoms produced are chiefly due to involvement of the central nervous system which is first stimulated and then paralysed. The special senses are usually affected most, and anomalies of vision and colour perception are commonly produced. At first white objects appear bluish, this is soon followed by xanthopsia or yellow vision which may persist for a few days and then disappear; blue seems green and violet is scarcely perceived. The usual dose producing these symptoms is 0.2 gm, the effect being probably on the cranial nerves. Smell, taste and hearing are sometimes affected. When large quantities of santonin are absorbed, twitchings and epileptiform convulsions, resembling those occurring in strychnine poisoning, are produced. In man convulsions rarely occur, but they are common in the dog and rabbit. The animal usually dies of asphyxia from stoppage of respiration. Santonin is chiefly eliminated in the urine partly unchanged and partly in the form of oxysantonin which gives the urine and sometimes the fæces a deep yellow colour. This colouration resembles that produced by anthracene purgatives, such as rhubarb, whose active principles are excreted in the urine. The two can be differentiated by the fact that by shaking the urine with a little lime water or barium water the santonin colouration remains in solution while the anthracene bodies are precipitated. Elimination of santonin begins quickly, but may go on for several days.

In vitro, ascaris are not killed even by saturated solutions of santonin in olive oil or castor oil. The parasites, however, become irritated and try to escape from the proximity of the drug. The irritant effect of the drug causes the parasites to move about in their efforts to escape, thus driving them into the colon whence they are expelled by a purgative. This irritant action can also be seen on the muscle tissue of leeches and worms; it is specific and is probably due to the presence of the lactone group though some believe that the anthelmintic action is in no way correlated with the structure of the lactone. It is not produced by santonic acid and is not apparent on the muscles of the vertebrates. The presence of dilute bile and alkali appears to increase markedly the toxic action of santonin on the parasites.

Santonin as an anthelmintic.

Santonin is considered the most effective drug against ascaris, with the possible exception of oil of chenopodium and hexylresorcinol. It is useless in intestinal tæniasis. Santonin acts best when combined with 3 or 4 grains of calomel and followed by a saline purgative six hours later. Calomel stimulates the flow of bile which enhances the toxic action of santonin on the parasites. Santonin is best prescribed in the form of a powder either as coarse crystals or as trochisci. In the form of coarse crystals as ordinarily sold it is preferable to the finely divided powder, as the latter is liable to produce toxic symptoms. Alkalies dissolve it forming soluble alkaline santoninates which are usually absorbed rapidly, and therefore this combination should be avoided.

Dosage for children and adults.

In determining the dosage for children a good rule is to give 1/6 grain for every year of age; for adults 1 to 5 grains according to the general constitution of the patient may be given. The preliminary preparation of the patient and after-purgative are most important considerations in santonin administration. The following procedure has been used successfully at the Calcutta School of Tropical Medicine:—

The patient is given his last meal at 5 p. m., no food being allowed after that. In severe infections it is advisable to keep the patient on light diet for 24 hours previous to administration of the drug. At 10 p. m. 3 grains (0.2 gm) of santonin combined with 3 grains (0.2 gm) of calomel are given. At 6 a. m. the following morning, ½ to 1 ounce saturated solution of magnesium sulphate or sodium sulphate is given. The stool should be examined for 72 hours after treatment for the worms and should be re-examined about 10 days later for ova; if negative the patient may be considered cured. The drug is toxic and not very effective and is being replaced by hexylresorcinol.

Idiosyncrasy.

As a rule no symptoms are observed, but nausea followed by vomiting may be produced. By using the above method 18 out of 19 cases were cured in one series. Santonin is a poisonous drug and idiosyncracy to the drug is common. It is, therefore, recommended to give 1/3 or 1/2 of the total dose the first time and if no sensitiveness is shown the drug may be repeated.

Some authorities give santonin combined with an equal or larger doses of calomel every morning for 2 or 3 days in succession and repeat the process every 3 or 4 days as long as eggs are found. This procedure is likely to lead to toxic effects. Some observers consider it inadvisable to use an oily purgative such as castor oil with santonin, as it is likely to increase absorption; others consider that it inhibits absorption.

Combination of santonin and chenopodium.

Maplestone and Mukerji (1931) reported very encouraging results by combining santonin with chenopodium in the treatment of roundworm infestations. The method employed by them consists in the administration of 5 grains (0.3 gm) of santonin in powder form and 1 ccm of oil of chenopodium in a capsule at the same time in the morning. A saline purgative is given after two hours. According to these workers santonin cures 60 per cent of adult and 48.2 per cent of children and the figures for combined treatment (santonin and oil of chenopodium) are 92.3 per cent and 54.8 per cent respectively.

The same workers (1938) working on the efficacy of oil of chenopodium and santonin opined that reduction in the amount of santonin has little effect compared with that which follows a reduction in the amount of oil chenopodium.

Santonin is a toxic drug and should on no account be prescribed unless the diagnosis is made by thorough examination of the stools. The practice of prescribing santonin on the mere assumption of parasitic infestation is to be deprecated.

Subcutaneous, intramuscular and intravenous injections of 'santonin-sodium' have been successfully tried against ascaris.

Other uses of santonin.

Santonin in doses of 3 grains for three days has been used in the treatment of amœbic dysentery and sprue, the yellow santonin being specially recommended in the latter condition. It has also been used in long-standing cases of diabetes associated with nervous disturbances particularly numbness of the extremities and pain of the sciatic or brachial plexuses. Such pains are sometimes relieved by such minute doses as 1/60 grain (0.001 gm) or less, three or four times a day. The urinary sugar may diminish and the nervous symptoms abate with doses of santonin gradually increased from 3 grains (0.2 gm) to 10 grains (0.6 gm) daily. Patients susceptible to the drug should be previously tested by giving ½ grain (0.03 gm) and it should not be continued for more than 10 days. Santonin also relieves the lightning pains of *tabes dorsalis* the initial dose for this purpose being 1/120 to 1/60 grain (0.5 to 1.0 mgm). It has been used in the treatment of epilepsy in doses of 2 to 5 grains (0.12 to 0.3 gm). In small doses of 1/60 to 1/6 grain (1 to 10 mgm) it has been recommended in the treatment of retinitis, amblyopia, and colour-blindness on account of its stimulant action on the retinal nerves.

Toxic effects.

Toxic manifestations are usually evident when the drug is rapidly absorbed and these, in their turn, depend on the conditions of the alimentary tract favouring or retarding absorption. Children are more susceptible and the fatal cases reported are entirely among infants. Adults as a rule are not seriously affected by doses less than 7 or 8 grains (0.5 gm). In infants under 3 years, death may occur with a one grain dose; the drug should therefore be used very cautiously in them. Repeated use of the drug is known to lead to tolerance.

Symptoms of santonin poisoning vary from slight manifestations of anomalies of vision to convulsions, coma and death. In mild poisoning digestive and ocular disturbances alone are observed, but in severe cases disturbances of the central nervous system predominate. Headache, vertigo, mental confusion, hallucinations, weakness, profound prostration, somnolence passing on to unconsciousness may develop. The diarrhœa is often profuse and the stool may contain blood; convulsions, coma and death may follow. The heart becomes slow and feeble and there may be syncope from the fall of blood pressure due to depression of the heart and dilatation of the blood vessels. The kidneys and urinary passages are irritated producing painful micturition, albuminuria and hæmaturia. Tremors, spasms of muscles, and loss of speech have also been noticed.

Treatment of poisoning.

Treatment of poisoning consists in rapid and thorough emptying of the bowels by purgatives and an enema. A central emetic like apomorphine may be given. Stimulants such as atropine, caffeine, or camphor, may be administered, and the patient kept warm. If convulsions are present repeated doses of chloral hydrate or inhalations of chloroform or ether may be tried. If respiration fails, artificial respiration should be resorted to.

Precautions and contra-indications.

It is advisable not to give santonin on an empty stomach as it is soluble in the gastric juice and may be absorbed. It is preferable not to give it with an oily cathartic. Fever is not a contra-indication to the administration of this drug.

Preparations.

Santonica with a santonin content of 1.5 to 3 per cent is used in ½ to 1 drachm (2 to 4 gm) doses as an anthelmintic.

A combination of santonin and bile called *santonin bile* has been prepared but it may produce toxic symptoms from rapid absorption.

Santoninum. Dose 1 to 3 grains; U.S.P. dose 1 grain (0.06 gm); in the French Codex 1½ grains as a single dose, the maximum dose given during 24 hours being 5 grains. *Chromosantonin* (N.O.) or golden santonin is a modification of ordinary santonin formed by exposure to sunlight. The dose is the same as ordinary santonin and it is stated to be useful in sprue and dysentery. *Trochiscus santonini* contains 1 grain in each. Dose 1 to 3. *Trochiscus santonini Co.* contains ½ grain of santonin and calomel each. Dose 1 to 2 grains.

Confectio santonini Co. containing santonin in combination with ginger, jalap, sulphur and senna is used in some of the children's hospitals.

Haustus santonini et olei ricini, contains 4 grains of santonin, half an ounce of castor oil, half an ounce of mucilago and peppermint water. It is prescribed on an empty stomach but symptoms of poisoning may occur.

Sodii santoninas. Dose 1/50 grain (1 mgm) cautiously increased to 1/20 or 1/10 grain (3 to 6 mgm) is used for systemic effects. As an anthelmintic 1/4 to 1 grain for adults preferably in salol-coated pills.

Calcii santoninas (N. O.). Dose 1 grain. It is a white tasteless insoluble powder.

Artemisine in doses of 1/600 grain (0.0001 gm) has been used as a gastric stimulant.

(5) Thymol and Allied Compounds

A number of compounds belonging to the stearoptene group, including menthol, borneol, camphor, and thymol have been tried as anthelmintics but only the last-named is effective. It has been shown that so far as these compounds are concerned, (*a*) the hydrogenation of benzene decreases their anthelmintic action, but increases their toxicity to the host, (*b*) the total destruction of the benzenoid structure leads to the formation of toxic compounds with no anthelmintic properties, (*c*) esterification of the phenolic hydroxyl group leads to formation of non-anthelmintic and non-toxic compounds, *e. g.*, thymotal.

(a) Thymol

Thymol was first tried in the treatment of hookworm infections by Bozzolo in 1879. Since then it has been extensively used with satisfactory results. In recent years, however, oil of chenopodium, carbon tetrachloride and tetrachlorethylene have largely supplanted this drug, for they are more effective and less dangerous than thymol and can safely be used for mass treatment and labour forces.

Sources.

Thymol or thyme camphor is contained in a number of essential oils from many plants, the chief amongst which is *Thymus vulgaris,* an evergreen shrub belonging to the Labiatæ family. In India thymol is manufactured on a commercial scale from *Cuminum cyminum* which contains large quantities of cumin oil whose chief constituent is cumin aldehyde which can be readily converted into thymol. The oil of *Carum copitum* known as oil of *ajowan* also contains not less than 40 to 50 per cent of thymol. The oil from *Monarda punctata* growing in America contains 60 per cent of thymol and *Origanum hirtum* growing in Crete contains 60 to 67 per cent

Chemistry.

The chemical formula of thymol is methyl-isopropyl-phenol and it is a stearoptene. It is a higher homologue of benzene. It occurs in large transparent crystals, slightly soluble in water (1 in 900) but freely soluble in fats, oil and alcohol. When it comes in contact with menthol, chloral, camphor, etc., it liquifies them.

Pharmacological action.

The action of thymol is similar to that of phenol; it has about one fourth the antiseptic power of phenol and in concentration as low as 1 in 10,000 kills free-living protozoa such as paramœcia. It is fatal to staphylococcus aureus in 0.5 per cent concentration, to typhoid and anthrax bacilli in 0.8 per cent and pneumococcus in 1 per cent. Growth of putrefactive bacteria is prevented by 0.1 per cent solution and 1.0 per cent is required to kill them. It inhibits the growth of moulds in 0.02 per cent solution.

Externally thymol has no action on the unbroken skin but it can exert its caustic or irritant action on mucous membranes. When taken internally in ordinary anthelmintic doses, it produces a feeling of well-being and comfort in the stomach; there is reflex stimulation of the peristaltic movements of the intestines. The presence of fatty constituents in the intestines promotes absorption of thymol and if this absorption is large it gives rise to systemic effects and produce fatty degeneration of the liver. It is, therefore, advisable to avoid fats, alcohol, glycerine, etc., when thymol is being administered.

Small doses of thymol administered orally produce reflex stimulation of the heart and the respiratory centre; large doses produce a marked depression and finally paralysis. The central nervous system is always affected to a certain extent. There is at first a slight stimulation of the cerebral cortex followed by depression which gives rise to narcotic effects. Headache, buzzing in the ears, dizziness, lowering of the body temperature and collapse follow when large quantities are absorbed.

Given by the mouth in doses of 15 to 30 grains (1 to 2 gm) most of the thymol is absorbed for none is found in the fæces. About half of the drug ingested is excreted in the urine partly in combination with sulphuric and glycuronic acids, partly unchanged and partly oxidised to a bivalent phenol-thymol hydroquinone. The greenish tint so often noticed in the urine after administration of thymol is due to the presence of this substance. The kidneys are irritated after large doses and albuminuria may result. When given in excessive doses it is also excreted in the bronchial mucous membrane and may produce inflammation of the lungs. The drug has no direct effect on the uterine movements but it may reflexly stimulate them by irritating the pelvic viscera.

In vitro thymol has a powerful action on earthworms. In 1 in 2,000 solutions the worms show signs of irritation and their movements are markedly increased at first, but they are soon paralysed and die in 10 to 15 minutes. In 1 in 10,000 solution they are killed in 1½ hours; and 1 in 20,000 they are rapidly paralysed but are not killed. Hookworms are killed by weak solutions of the drug.

Anthelmintic effects.

There is no unanimity of opinion about the effective dose of thymol. Some authors have recommended as much as 120 to 150 grains in divided doses of 25 to 30 grains, while others consider such large doses are neither necessary nor justifiable. They do not advise more than 60 grains of thymol in a day and recommend not to repeat it in less than a week. In pregnant women the dose should not exceed 30 grains, and in cases of extreme debility from old age, heart disease, chronic diarrhœa and dysentery the dose should be

small and divided. The drug should at once be stopped if untoward symptoms appear. Ashford and King recommend the following scale of dosage:—

Dosage.

Under 5 years 8 grains (0.5 gm); 5—10 years 15 grains (1.0 gm); 10—15 years 30 grains (2.0 gm); 15—20 years 45 grains (3.0 gm); 20—60 years 60 grains (4.0 gm); over 60 years 30—45 grains (2 to 3 gm).

Method of administration.

It is customary to give the drug in two or three divided doses, of 15, 20 or 30 grains each at intervals of 1 to 2 hours followed by a strong saline purgative. It is better not to give coarse crystals as these are likely to pass through the bowels unchanged, probably without coming in contact with the parasites. The drug also has a tendency to pack together in hard lumps if given in capsules and it is preferable to give it mixed with sugar of milk or bicarbonate of soda. In case of children who are not capable of swallowing capsules, thymol is best given in powdered form suspended in simple syrup or mixed with chocolate.

Preparation of patient.

The preparation of the patient is important when thymol is being given as an anthelmintic. The following procedure is recommended (Ashford):

The patient is given a light meal and one ounce of saturated solution of magnesium or sodium sulphate the night before. At 8-30 a.m. the following morning 30 grains (2.0 gm) of powdered thymol are given in capsules; at 10 a.m. another similar dose is given; at 11 or 12 o'clock, 1 ounce of saturated solution of magnesium sulphate is given. No breakfast is allowed and food is only permitted after the bowels have moved. The patient should remain in bed and should use a bed pan.

Effectiveness.

Thymol is the classical drug for the treatment of hookworm infections, but more than one course of treatment is often required to produce a cure. In one series 68.6 per cent. were cured in 30 days and 94.4 per cent in three months (presumably 4 to 8 treatments). The drug is believed to be more effective against mature worms. Fourteen to fifteen days after infection the worms develop a buccal capsule and attach themselves to and feed on the mucous membrane. From this time onward they are exposed to the action of the drug and treatment should then be given. In another series, investigators succeeded in removing 95.7 per cent of necators and 57.9 per cent of ankylostomes with a single dose of 60 grains (4.0 gm) of thymol. Even with such large doses as 180 grains (12.0 gm) the drug is less effective against ankylostomes than other available anthelmintic. It is still regarded by some as the best anthelmintic against hookworm, since even after 60 grain doses no death occurred.

Maplestone and Mukerji (1940) after a comparative study, conclude, that though the efficiency of thymol is undeniable, yet considering the cheapness, safety of patient, simplicity of adminstration, and proved efficacy against hookworms, tetrachlorethylene is the drug of choice.

Effect on other nematodes.

The effect of thymol on other intestinal nematodes is distincly inferior to its effect on hookworms. Ascaris, trichuris and oxyuris are not very vulnerable to its action. Tapeworm segments are expelled but the removal of the heads is very doubtful. Thymol has also been used in trichiniasis and the parasites are expelled when they are present in the gut. Good results have also been obtained even when the parasites have lodged in the muscles. A 6 per cent solution of the drug in oil has been given intramuscularly in doses of 2 to 3 ccm injections being given daily. No toxic symptoms are observed and there is marked improvement in the patient's condition. Trichiniasis can be prevented if treatment *per os* is begun within 24 hours of the ingestion of infested meat, with 2 grains (0.13 gm) of thymol repeated three or four times and followed by a purge.

Other uses.

Thymol in concentrations of 1 grain in an ounce is used for spraying the nasal mucous membrane in catarrhal affections. For general disinfection a solution of 1 in 200 (one tablespoonful in 5 pints) should be used; thymol has also been used as a paint in ringworm, eczema and various skin diseases. In amœbic dysentery considerable benefit is obtained by colonic irrigation with 1 in 2,000 solutions in conjunction with emetine bismuth iodide. In flagellate diarrhœa, an enema with 1 in 2,000 solution and 4 to 8 grains (0.25 to 0.5 gm) of thymol by the mouth are recommended. A 5 per cent solution in alcohol and 2.0 per cent in cinnamon oil used as a paint to control infestations of the skin by fungi. One to 2 grains (0.06 to 0.13 gm) are given 3 or 4 times a day in typhoid fever with tympanitis.

Toxic effects.

In large doses thymol produces symptoms resembling carbolic acid poisoning. There is usually acute pain over the epigastrium, due to the caustic and irritant action of the drug on the mucous membrane of the stomach; nausea and vomiting follow and the patient be-

comes prostrated. Giddiness, roaring sounds in the ears, drowsiness, etc., commonly occur; there is often salivation and cyanosis, and the patient may become unconscious. The temperature has a tendency to fall below normal and both the pulse and respiration are slowed; skin rashes may appear; the urine may become scanty; abortion may follow in pregnant women. With very large doses collapse may be rapidly produced and death may occur in a few hours. The intensity of symptoms depends on the amount absorbed, and the presence of solvents like fats and alcohol strongly increases the tendency to produce intoxication. A purgative given a few hours after the drug prevents intoxication by washing it out of the gut. Sometimes the intoxication is delayed and sets in several days later when all the symptoms except weakness have disappeared.

Treatment of poisoning.

In poisoning, the stomach should be rapidly washed out with warm water. Demulcents may be given to prevent corrosion of the mucosa. Cardiac and respiratory stimulants, *e. g.*, 1/120 grain (0.005 gm) of atropine and 1/30 grain (0.002 gm) of strychnine should be given. Hot coffee is useful in overcoming weakness. Alcoholic stimulants and tinctures are contraindicated.

Precautions and contra-indications.

Thymol should be given with caution when there is anæmia, nephritis, advanced cardiac disease, pregnancy and debility due to old age. Patients suffering from chronic diarrhœa and dysentery also take it badly.

Thymotal.

Attempts have been made to lower the toxicity of alkyl phenols by converting them into non-irritant esters, such as carbonates, carbamates, etc. Thymotal or thymol-carbonate is prepared by passing phosgene gas through a concentrated solution of thymol in an aqueous solution of caustic soda. It was at one time considered to be a very effective remedy against ankylostomes, but later it was found to be absolutely useless even in 40 grain doses. Although these esters are broken up in the alimentary tract by hydrolysis forming the original hydroxyl compounds, they do not attain sufficient concentration to be effective anthelmintics.

Isothymol.

Isothymol or *carvacrol* was synthetically prepared from turpentine during the Great War when the supply of thymol from Germany was stopped. Chemically carvacrol is an isomer of thymol and its pharmacological action bears a close resemblance to thymol in many respects. The anthelmintic action of carvacrol was tested by Sollmann on earthworms with fairly satisfactory results. The International Health Board reported unfavourably on its efficiency against hookworm. Caius and Mhaskar (1924) tried carvacrol in 20—60 minim. doses with promising results. They found it to be a good anthelmintic but decidedly inferior to thymol. It is also more irritant and somewhat more toxic than thymol.

Preparations.

Thymol disinfectant (Martindale). It is a potent antiseptic; when employed as a general disinfectant, 1 in 200 solution in water should be used.

Liquor thymol is made of 1 part of thymol in 800 parts of warm water; used as an antiseptic gargle well-diluted.

Mistura oleobalsamica. Dose is 1 to 4 drachms in water as a carminative stimulant.
Pastilli thymol. Contains 1/32 grain of thymol in each.

Pigmentum thymol. It consists of thymol 1 part, ether 10 parts and spirit 5 parts or may also be prepared with petroleum oil; useful in ringworm of the scalp.

Thymaglycine. It is a palatable preparation containing sodium benzoate, menthol, essential oils, glycerine and thymol water. It is given in rhinitis, pharyngitis, quinsy, etc., or as a spray for throat and nostrils, diluted 1 to 3 in water. For colitis in children 5 to 10 min in paraffin or water are given.

Glycothymoline. It consists of potassium carbonate, sodium benzoate, sodium borate, sodium salicylate, thymol, menthol, glycerine and alcohol coloured with cochineal. It is frequently prescribed as an intestinal antiseptic in doses of ½ to 1 drachm and also as a gargle.

Unguentum thymol. Prepared in strength of 20 grains (1.3 gm) in an ounce of soft paraffin useful in eczema. A compound ointment has boric acid and zinc oleate in addition.

Thymol carbonate (Thymotal). It is a colourless and tasteless crystalline powder employed in ankylostomiasis in doses of 5 to 15 grains (0.3 to 1.0 gm).

Thymoform is obtainable in the form of lozenges containing thymol and formic aldehyde in a cane-sugar basis.

(b) Beta-Naphthol

Beta-naphthol has been used as an anthelmintic for quite a long time. As early as 1904, Bentley used the drug against hookworm in Assam and later in 1908, Burkitt and Drummond tried it with excellent results. The chief merits claimed on its behalf are that it is not unpleasant to take, it is more satisfactory for routine use and is one-tenth the price of thymol. Caius and Mhaskar reported very favourably on the drug and recommended it as the safest and most efficacious anthelmintic for hookworms, but subsequent work has failed to substantiate their claims. With the advent of carbon tetrachloride and oil of chenopodium beta-naphthol is gradually receding from the field though it is still used in some places.

Source and composition.

Naphthol is derived from the hydrocarbon naphthalene by the substitution of a hydroxyl for one of the hydrogen atoms. There are two naphthols—the alpha- and the beta- naphthols —the former is very toxic and is not used in medicine.

Beta-naphthol occurs in colourless glistening crystals of pale buff-coloured lamellæ. It has a faint phenol-like odour and has a pungent hot taste; it is very slightly soluble in cold water but dissolves readily in boiling water. The drug is of uniform quality and does not readily change or deteriorate though it is better kept in amber-coloured bottles.

Pharmacological action.

Externally beta-naphthol is irritant to the unbroken skin and may be absorbed therefrom producing toxic symptoms. It produces inflammation and corrosion of mucous membranes and raw surfaces. Beta-naphthol can be taken by healthy individuals in fairly large quantities without producing any untoward symptoms. As a rule, a light degree of warmth is felt in the stomach, but there is no burning pain, nausea and vomiting as in the case of thymol. Beta-naphthol is sparingly soluble in saliva and in the gastric juice, hence its absorption from the stomach is slight; it is more readily absorbed from the small intestine. The peristaltic movements of the intestine are increased and some purging results. Large doses produce an irritant action on the gut and produce nausea and vomiting.

The heart is reflexly accelerated at first, but later it becomes slower which is due to direct action on the cardiac muscle. In large doses, it has a toxic effect on the red blood corpuscles, especially in patients with active or latent malaria. The corpuscles may be destroyed in sufficient quantities to produce hæmoglobinuria, jaundice, and severe anæmia. Large doses depress the respiration probably from depression of the respiratory centre. Therapeutic doses have no effect on the central nervous system, but after absorption of large amounts, it produces giddiness and convulsions followed by paralysis and coma. Changes in the retina and opacity in the lens have been produced in some cases, after its use.

Beta-naphthol is rapidly excreted in the urine and during its excretion it may produce inflammation of the kidneys, even when the doses taken are not large. It is excreted in combination with glycuronic and sulphuric acids and imparts to the urine a dark brown or mahogany red colour. Although salivation is produced, it is not excreted in the saliva.

Dosage.

There is great diversity of opinion about the toxicity and efficacy of beta-naphthol as an anthelmintic. The dosage recommended varies from 30 to 90 grains (2.0 to 6.0 gm), on three successive days until the total quantity has been given. Such large doses often produce destruction of the red blood corpuscles; doses of 30 grains (2.0 gm), on the other hand appear to be too small. According to Caius and Mhaskar, the drug is quite safe and effective in doses of 50 grains (3.3 gm) in a single dose, and 60 grains (4.0 gm) divided into two doses. There is again no definite rule about the use of the pre-purge. The omission of the preliminary purge is not attended by any fall in anthelmintic efficiency and the after-purgative is often unnecessary in as much as the drug itself has got a purgative effect. It is, however, better to use both the pre- and post-purgative, for this procedure decreases the chances of production of untoward effects.

Efficiency.

Beta-naphthol has been found by nearly all workers with the exception of Caius and Mhaskar to be less efficient than thymol, and certainly less so than oil of chenopodium or carbon tetrachloride. Usually at least four or five treatments are necessary before a cure is effected. Its action on ascaris and trichuris is no more promising than its action in hookworms. Smillie in Brazil removed only 52 of 124 ascaris (41 per cent) as compared with 91 per cent of 454 cases removed by chenopodium. Tæniæ are also very little affected by it.

Toxic effects.

The toxicity of naphthalene bodies in man varies widely. Naphthalene has been taken in 75 grains (5.0 gm) doses without ill effects, while much smaller quantities have produced severe gastro-enteritis. Deaths have been reported from the external use of a mixture of 1 part of beta-naphthol and 2 parts of camphor. In man, restlessness followed by con-

vulsions and coma with stertorous breathing, have been seen even in non-fatal cases. Naphthalene and beta-naphthol are toxic drugs, 2 drachms (8 gm) causing death in a rabbit; in the dog the fatal dose is 1.0 gm per kilo; cats die of convulsions after 1.5 grains (0.1 gm). Large doses produce a burning sensation in the epigastrium, diffuse abdominal pain, nausea, vomiting, diarrhœa, or even dysentery like symptoms, spasm of the face muscles, weakness and fever. Micturition is painful, the urine becomes very dark, scanty, and shows the presence of bile, albumen and casts; suppression of urine may supervene, producing uræmia. The spleen, the liver and the kidneys are enlarged and hyperæmic, and the gall bladder is distended. Death may occur from convulsions or gradual failure of respiration, the heart goes on beating after the respiration has stopped. All these symptoms are more readily produced in malarial patients. The Porto Rican Anæmia Commission tried the drug extensively and found that 83.3 per cent of their cases who took it suffered from albuminuria. Caius and Mhaskar (1921) noted that all their cases of albuminuria and hæmoglobinuria occurred with doses over 60 grains administered for several days. With small doses no such symptoms were produced.

Precautions and contra-indications.

The urine of patients should be always examined before administering the drug, and if the kidneys are damaged it should be given with caution. Large doses should not be given to those who are suffering from or have suffered from malaria.

Preparations.

Naphthol (B.P.) and beta-naphthol (U.S.P.X.). Dose 15 to 30 grains (1 to 2 gm), but 40 to 50 grains in single or divided doses is considered quite safe. It is usually given in the form of cachets or pills or in the form of an emulsion in oil. *Betol* or *beta-naphthol salicylate* (N.O.)[1] has no anthelmintic action. Dose 4—8 gr, used in the form of cachets or pills or as emulsion in milk in rheumatism, cystitis and intestinal catarrh. *Benzonaphthol* or *beta-naphthol benzoate* (N.O.) has also no vermicidal action, used as diuretic and intestinal antiseptic. Dose 15 grains (1.0 gm). *Beta-naphthol petroxalin* (N.F.) 10.0 gm of beta-naphthol dissolved in 90 gm of liquid petroxalin used as a parasiticide application. *Naphthalenus* (N.O.) has been tried against hookworm but has no anthelmintic action. Dose the same as beta-naphthol. *Naphthalene tetrachloride* was tried in 8 grain (0.5 gm) cachets every 4 hours in colitis.

(6) Resorcinol Derivatives

A number of synthetic compounds have been prepared and some, the isohexyl derivatives of either resorcinol or phloroglucin, are promising.

(a) Hexylresorcinol

Properties.

Hexylresorcinol is 1 : 3 dihydroxy -4- hexylbenzol. It is a white, waxy, crystalline substance and is quite stable. It is sparingly soluble in water (1 in 1,700) but it readily dissolves in alcohol, ether, chloroform and vegetable oils. Hexylresorcinol is considered to have highly germicidal properties, the phenol co-efficient varying between 46 and 52. It has been used as a mouth wash, gargle, nose and throat spray in concentration of 1 in 4,000. It has been administered in gelatine capsules in doses of 0.45 to 0.6 gm three times a day, as an urinary antiseptic. The drug has been continued for as long as ten weeks without showing any deleterious effects. For children, a 2½ per cent solution of the drug in olive oil under the name of Caprokol (N.N.R.) has been prescribed two or three times a day. It has been highly spoken of in cystitis and pyelitis and appears to be well tolerated.

It is said to be a safe and effective anthelmintic in nematode infestations, both round worms and hook worms. It is given in doses of 15 grs in adults, in hard gelatine capsules, to avoid irritation of the mouth. According to some, even given in this way, there is danger of necrosis of the gastric mucosa when the gelatine dissolves. As the drug combines with the food, dieting is essential.

Pharmacological action.

Crystalline hexylresorcinol has irritant properties and is liable to produce a burning sensation in the mouth. The irritating property of the drug can be overcome by dissolving it in olive oil but this reduces its vermicidal power. A solution of 0.1 per cent of hexylresorcinol *in vitro* killed pig ascaris in 2 minutes while it took more than 20 minutes to

be effective in 3 per cent olive oil solution. Repeated administration to dogs is known to have produced petechial hæmorrhages in the buccal and gastric mucous membranes. The drug is, however, precipitated by proteins, and probably on this account it does not penetrate deeply into the tissues. When given with alcohol absorption is facilitated. Experiments on dogs show that about 27 per cent. of hexylresorcinol taken by the mouth is excreted in the urine and 67 per cent is eliminated in the fæces.

Anthelmintic properties.

The anthelmintic property of the drug has been noticed only recently. Lamson and others in America were the first to study the effect of hexylresorcinol as an anthelmintic. They found that the drug in large dilutions was lethal to pig ascaris *in vitro*. It removed 100 per cent of the ascaris in 16 out of 17 dogs as shown by autopsy, and it removed 90 to 100 per cent of these parasites in 20 patients when given in 1 gm doses in gelatin capsules. This was followed later by more systematic investigations into the effectiveness of the drug in human ascariasis. The drug was administered in hard gelatin capsules to avoid the local irritant effect, the maximum dose being 1.0 gm for adults, magnesium sulphate being given two hours after. They succeeded in curing 7 out of 10 cases; the egg reduction amounted to 96.4 per cent in a total of 5 cases, all of whom were cured. The reduction was cent. per cent. if the purgative was deferred for 24 hours. Food, shortly before or after the treatment, greatly reduces its efficacy. Lamson and his co-workers (1932) in evaluating the comparative results of hexyl and heptylresorcinol considered the former to be a better anthelmintic. Lamson, Brown and Ward (1932) consider a single dose of hexylresorcinol to be capable of removing more than 90—95 per cent of ascaris, 80—85 per cent of hookworms and 40—45 per cent of trichuris. Maplestone and Mukerji (1932) using the drug in hard gelatin capsules, under strict fasting conditions, obtained a cure rate of 66.6 per cent and an egg-reduction of 94 per cent. in 21 cases. In hookworm infestation in 26 cases the corresponding figures were 7.7 and 71.4. In ten cases of tæniasis no head was recovered after giving the drug but five reported no recurrence 3 months later.

Method of administration.

Dosage.

Before administering the drug, proper dietary preparation is necessary. In the evening previous to the commencement of the treatment, only milk and bread should be given. Early next morning hexylresorcinol is given in hard gelatin capsule in doses of 1.0 gm with about 2 ounces of water to help in swallowing the drug. No food is allowed for 4 to 5 hours and alcohol is forbidden. Reaction between the drug and gelatin can be avoided if the capsules are filled immediately before they are administered. Sugar-coated pills each containing 0.2 gm are available and they are unaffected by climatic conditions. They are quite convenient to use except that in children they may cause a superficial burn in the mouth if they are chewed, on account of the protein-precipitating property of the drug when it comes in contact with the mucous membrane. In the majority of cases, hexylresorcinol exerts a cathartic action and the patient has watery stools after it. A dose of magnesium sulphate should however be given next morning, *i. e.*, 24 hours after the dose. The toxic symptoms observed are negligible. Some of the patients may complain of pain in the epigastrium but this is very rare, even with doses as high as 2 gm for a single administration. Repetition of the dose even at short intervals in maximum quantity does not produce any toxic symptoms. Hexylresorcinol, so far as is known has proved of value in ascariasis; in ankylostomiasis and other helminthic infection, its superior efficacy over other drugs has yet to be worked out. Probably in hookworm infection, in anæmic and debilitated subjects it may be a safer drug than the other remedies in use.

Post-purge.

Hexylresorcinol is the nearest approach to an ideal anthelmintic drug and is therefore the drug of choice in ascaris and other infections. Its toxic effects are few and are mild; there are no cumulative toxic effects; contraindications are few. The drug can be administered when the patient is up and about. Its efficiency against ascaris equals any of the known drugs and it is also effective against hookworm though not equal to tetrachlorethylene. It is administered in maximum doses of 1.0 gm (15 grains). Two doses administered at intervals of two weeks in ascaris infection cure the majority of cases. A single dose

expells a large number of hookworms. When these two infections are present together it is an ideal drug.

The dose in children is 0.1 gm for every year of age up to 10 years.

In tapeworm infections it is also effective and it has been successfully used against *Fasciolopsis buski* and whip worms. In oxyuric infections it is given by the mouth combined with high enemata of hexylresorcinol solution.

The pills should not be chewed as they set up irritation of mouth; they should be put far back on the tongue and swallowed. The drug should be given on empty stomach and food should be withheld for four hours after administration. It is also advisable to give a saline purgative the evening before, but an after purgative is not necessary except 24 hours after the drug to expell dead worms. The following routine of treatment is prescribed for hookworms and round worms.

(1) The day before, magnesium sulphate is given in the afternoon (castor oil is contraindicated). Patient is kept on liquid diet the whole day.

(2) On the day of administration, no food or drink is given till the drug is given. Total dose is given at one time. No food or drink for four hours after, but fruit juice may be allowed with plenty of sugar. Light food to be taken that day.

(3) For tapeworms three full doses on alternate days followed by a saline purgative are best. In enterobius infections, the drug is combined with, soap enema or high enema with 1—2,000 solution of the drug which is retained for 20 minutes if possible. After enema wash and dry anal region to prevent irritation.

(b) Heptylresorcinol

On account of the results of hexylresorcinol in the treatment of helminthic infestations other alkyl resorcinols have also been used. Heptylresorcinol is one of them. Locally, it produces a smarting burning effect on the tongue and is irritant to the buccal and gastric mucous membrane. About 90 per cent. of heptylresorcinol is excreted in the fæces. Lamson and his co-workers (1932) in a comparative study of the effect of the two resorcinols in helminthic infections found that hexylresorcinol seemed more effective against hookworm, ascaris and trichuris than heptylresorcinol. The average egg-reduction in hookworm disease with 1.0 gm. doses of heptylresorcinol was 55.2 per cent., in ascaris 73.5 per cent and in trichuris 35.8 per cent.

Hookworm infestations proved refractory towards heptylresorcinol dissolved in olive oil. In a series of 23 cases of hookworm infection treated with this drug; six were treated with olive oil solutions of heptylresorcinol, but this proved ineffective. The crystalline drug in gelatin capsules was later tried, which proved partially successful; trichuris infection proved refractory.

Heptylresorcinol is generally given in hard gelatin capsules in doses of 1.0 to 1.5 gm preferably in the morning on an empty stomach and food is withheld for 4 to 5 hours. Solution of the drug in olive oil greatly lowers its efficacy. A purgative should be prescribed several hours later. The drug has also been prescribed in gelatin capsules coated with salol. This prevents liberation of the drug in the stomach so that it may reach the parasite in the intestines in an effective concentration. The results of treatment are more favourable in hookworm infection than in ascariasis or tapeworm infestations.

Hexylresorcinolis (tablets 0.2 gm.), *Liquor Hexylresorcinolis* (1 in 1,000).

(7) TRIPHENYL METHANE (ROSANILINE) DERIVATIVES

The compounds most interesting from medical point of view are those which result from the introduction of amino groups forming pararosaniline. Three closely related compounds thus formed are gentian violet, crystal violet and methyl violet. Gentian violet is probably a mixture of methyl violet and crystal violet.

Gentian violet was introduced as an antiseptic for infected wounds, mucous membranes and serous surfaces. It is particularly useful in empyema and arthritis in which *staphylococci, Ps. aeroginosa* and *Cl diphtheria* are causative agents. It also has an anthelmintic action.

Gentian Violet in Helminthiasis

This drug has now been tried extensively but is still in experimental stage. It has been found to be specially effective in the treatment of enterobius infections. As compared with hexylresorcinol enemas it is simpler to administer and more effective. It is also effective against *strongyloides* and has been successfully tried against *Clonorchis sinensis.*

The drug is given in the form of coated tablets which are designed to dissolve in four, one-half and one hours after ingestion. There are few serious toxic effects though nausea, vomiting, abdominal pain, headache and dizziness have occured in some patients. These effects, however, soon pass off after the drug is stopped and the treatment can be resumed after a few days. The drug should not be given, when ascaris infection coexists with enterobius infection. It is contraindicated in disease of the heart, kidney and the gastrointestinal tract. Alcohol is forbidden during the course of treatment with this drug.

Mode of Administration.

Enteric coated tablets are sold each containing ¼ gr (0.016 gm) and ½ gr (0.032 gm). For children weighing less than 100 pounds the proceedure is to give half grain three times a day, one hour before meals for fourteen days. The treatment is discontinued for seven days and the course of fourteen days is repeated. For adults weighing more than hundred pounds, 1 gr (0.06 gm) tablets are given three times a day one hour before meals for seven days; rest for seven day and then the course is repeated, altogether fifty grains being taken. For strongyloides and clonorchis infection the drug is taken in doses of one grain three times a day for fifteen days. In refractory cases, tablets with one and one-half hour coating are used. Faust (1939) suggested intravenous injections of 0.5 or 1.0 per cent solution in distilled water in refractory cases, and in bronchial and extra-enteric strongyloides infections. Injections were given in doses of 20 to 25 ccm every third day. Violet colouration of the skin and fever may occur but these pass off. Injections must be given slowly. No untoward effects have been observed so far.

Sostol.—This is a yellow crystalline powder and is the hydrochloride of an acridine compound. It is soluble and is said to be specially effective against diphyllobothrium and hymenolepis infections. The patient is prepared in the usual manner and sostal pellets, 6 of 0.1 gm (1½ gr.) are given first thing in the morning on an empty stomach; three hours after, a dose of castor oil or magnesium sulphate is given. Children under six years receive 1 to ½ pellets, and children from five to eight, 4 pellets.

(8) MISCELLANEOUS ANTHELMINTICS

Intestinal antiseptics.

Intestinal antiseptics. Some of the compounds which show well marked disinfectant properties in the gastro-intestinal tract, such as thymol and beta-naphthol, have proved excellent anthelmintics. This observation opened up the possibility of other intestinal antiseptics behaving in a similar manner. With this idea in view, a number of reputed intestinal antiseptic drugs have been tried to determine their vermicidal properties. It has been found, after a large series of experiments conducted by workers in different parts of the world, that these drugs, with the possible exception of some of the propenyl derivatives of phenol and salicylic acid esters, are useless as far as their anthelmintic activity is concerned. Some of these are positively dangerous to the host instead of being in any way inimical to the parasites.

Potassium permanganate in maximum doses, bismuth carbonate, kaolin, naphthalene, phenocoll, methylene blue, guiacol carbonate, creosote, izal, salicylic acid, salol, acetyl-salicylic acid (aspirin), salicylic ester of beta-naphthol (betol), the crude phenols, etc., have all been tried and found to be useless.

Methyl salicylate, a volatile ester of salicylic acid has, however, well-marked vermicidal properties. In doses of 30 to 60 min (2 to 4 ccm) it has been found to be very effective against hookworms, but has little or no effect on ascaris and trichuris. Methyl salicylate is present in oil of wintergreen (oleum gaultheria) in large quantities (99 per cent.). It has been given in 30-60 min doses with castor oil and gum acacia with satisfactory results.

A number of propenyl phenols occur in nature, *e. g.*, anethol, eugenol, etc. These are fairly good anthelmintics and do not possess the toxic properties on the host, of the crude phenols.

Purgatives.

Purgatives. These drugs have got very little vermicidal action but they irritate the gastro-intestinal tract and in this way bring about a vermifugal effect. Magnesium sulphate, calomel, phenolphthalin, oleum ricini, oleum tiglii and aloes have all been tried at some time or other by different workers, but they have no vermicidal properties. Whipworms (trichuris) have been successfully treated with two ounces of castor oil on four successive days, followed by an enema of 2 litres of 0.5 per cent solution of sodium carbonate with one ounce of kaolin added to it. The patient is kept on a low diet and is also given one ounce of kaolin daily on each of these days. After four days of treatment the enema is continued for two days more.

The heavy metals.

Heavy metals. A number of other drugs have been used for their anthelmintic effects in man and animals. Copper sulphate has chiefly been used in veterinary practice, especially in infection with *Haemonchus contortus,* the stomach worm of sheep. The arsenical compounds such as stovarsol have been given but their vermifugal effect is doubtful. Injections of organic arsenicals and tartar emetic have been found to have some effect on clonorchis infections in man. Salvarsan and sulpharsenol injections have been given in oxyuris and guineaworm infections with success in some cases. Mercurochrome has been given intravenously against *Schistosoma japonicum* in man in 1.0 per cent. solution, with some degree of success.

E. ribes and E. robusta.

Embelia ribes and *E. robusta* family Myrsinaceæ had the reputation of vermicidal action, but they have been shown to be quite ineffective against hookworms, round worms and whipworms. The chief constituent of the fruit is embelic acid, which occurs in golden-yellow crystals, resin and tannin. Anthelmintic dose 1 to 4 drachms (4 to 10 gm) of the powdered drug.

Azadirach.

Azadirach. The bark and root of *Melia azadirachta* has been used as an anthelmintic against roundworms. The active constituent of the drug is margosic acid and a resin. In India and in the southern United States, the fresh bark of the root is employed to expel ascaris. The powdered bark is given in doses of 20 grains (1.3 gm), or the decoction (made by boiling 2 ounces of the bark in a pint of water until reduced to half) is given in one tablespoonful doses hourly or every second or third hour; a purgative is given after the second or third dose. The oil has no anthelmintic properties. In excessive quantities the drug produces dizziness, purging and collapse.

Corsican moss.

Corsican moss (helminthochortan) is a mixture of various marine algæ and is used in France and Italy to expel ascaris. It is best given as a sweetened decoction with milk in a single dose before breakfast. Dose for a child under two years is 15 to 45 grains (1 to 3 gm); of 10 years 3 to 4 drachms (12 to 15 gm). It is sometimes combined with santonin or santonica.

Mucuna or Cowhage is the fruit of *Mucuna pruriens,* a climbing plant of tropical America. The pod is covered with stiff hairs which produce intense itching. Cowhage acts mechanically, the hair piercing the parasites. The pod is dipped in molasses and the hairs are removed by scraping. Dose is one teaspoonful for a child and one tablespoonful for an adult. The drug has been used both against roundworms and tapeworms, but it is too disagreeable to take and too uncertain in its action. *Mucuna.*

Butea frondosa has been used as an anthemintic against roundworms but opinions differ regarding its efficacy. The seeds have been analysed and they contain 16 per cent of a fixed oil called *moodooga oil,* small quantities of resin and large quantities of a watersoluble albuminoid. There are no active principles of the nature of alkaloids, neutral principles or glucosides. The powdered seeds have been used as an anthelmintic in doses of 30 to 60 grains (2.0 to 4.0 gm), but they are unpleasant to take and often produce pain in the stomach, vomiting and giddiness. The oil and the resin were separately tested against hookworm and ascaris. The alcoholic extract, the oil and resin had no effect, but freshly powdered seeds produced good results in ascaris infection. Old worm-eaten seeds met with on the market are inactive. *Butea frondosa.*

Ficus laurifolia has been recommended as an anthelmintic against whipworm in man in doses of 15 to 30 gm. The latex of this plant has definite anthelmintic properties and is worthy of trial. The active principle is unknown though it probably resides in the solid portion of the latex. In its natural state, the latex forms a perfect emulsion of rubber and resinous substances; it contains an albumin and substance of fixed composition yielding ammonia, which is probably responsible for its anthelmintic activity. The latex unfortunately readily ferments and will only keep for 4 to 5 days, though in an ice box it has been kept for several months. The drug is very effective against whipworms (*Trichuris trichiura*). It may produce toxic symptoms, colic, nausea, vomiting, muscular cramps, delirium, syncope, urticaria, rectal and vesical spasms, and partial suppression of urine. *Ficus laurifolia.*

Quassia is the wood of the trunk and branches of *Picraena* or *picrasma excelsa. Picrasma quassioides* Benn is a common shrub growing in the sub-tropical Himalayas. The active principles of quassia are substances called picrasmin and quassin. *Quassia.*

Quassia is poisonous to many insects and is used as an insecticide. It has been used as an anthelmintic against threadworms for a long time. Caius and Mhaskar by giving 5 ounces of an infusion of the drug prepared by adding 1 ounce of finely powdered root to a pint of boiling water, failed to cure any hookworm case. It has, however, been found effective in threadworms especially in children; generally the decoction is given in the form of an enema, but quassia suppositories containing ¼ grain (0.016 gm) of the extract with a gelatin basis, may also be prescribed. Two grains of the extract of quassia in the form of a pill taken three times daily along with some purgative are also given to clear out threadworms.

Spigelia marilandica or pinkroot is a plant belonging to the family Loganiaceæ which is widely spread all over the southern parts of the United States. It was formerly considered as a powerful anthelmintic especially against ascaris. *Spigelia marilandica.*

The constituents of the root are *Spigeline,* which is a volatile alkaloid resembling nicotine and conine, a bitter principle soluble in water, a volatile oil and resin.

Very little is known about the action of this drug. The alkaloid is a gastro-intestinal irritant, and in poisonous doses it produces convulsions. It is absorbed slowly from the gut and if combined with a purgative it does not produce any toxic effects. Sollmann (1918) tested its activity on earthworms but his results were not promising. Pinkroot is chiefly used as a vermifuge against roundworms and is said to be quite effective against them. Careful experiments on animals have shown that it has no marked anthelmintic action against any of the parasites.

Chrysanthemum cinerariae. The active principle is said to be a good parasiticide, it is non-toxic and non-irritant. It is said to be useful in ascaris, trichuris, threadworm and tapeworm infections. *Chrysanthemum cinerariae.*

Rhumnus cathartica. A syrup made from the berries with jalap is said to be useful in oxyuriasis. *Rhumnus cathartica.*

Allium sativum. The allyl compounds contained, produce complete paralysis of ascaris *in vitro,* but their anthelmintic effects in man are doubtful.

Geraniol. *Geraniol* in doses 0.3 ccm per kilo body weight has been tried in dog but has no effect. Various species of *Cambretium* occurring in Brazil, extracts of *C. quadrangulans,* essential oils of *Tageterminulus* and *Kyllinga odorata* were quite ineffective.

Acalypha indica, Petroselinum sativum and *Quisqualis indica* have no anthelmintic properties.

Rotylon. *Rotylon.* It is an aromatic combination, insoluble in water, but soluble in alkalies, manufactured by Bayer Meister Lucius Ltd. The drug is set up in capsules each containing 0.4 gm. When taken in liquid form it produces a burning sensation in the tongue which is followed by one of prolonged anæsthesia. The anthelmintic properties of this drug are under investigation.

Vernonia anthelmintica. *Vernonia anthelmintica.* Powdered seeds in doses of 30 to 60 grains have a weak vermifugal action against ascaris and a more powerful action against oxyuris. The bitter substance isolated, in doses of 3 to 10 grains, has a weak vermifugal action against ascaris and a decided action against oxyuris. When combined with calomel and followed by magnesium sulphate the vermifugal action is considerably enhanced. The drug has no action whatsoever against the hookworm and tapeworms. The anthelmintic properties of the drug against ascaris and even against oxyuris are weaker and in no way comparable with some of the other compounds now in the British Pharmacopœia.

Phenothiazine. Phenothiazine (Thio-diphenyl-amin) has been recommended by Manson Bahr (1940) as an effective anthelmitic in ascaris and enterobius infections. This thiazine dye is fine smooth pale yellow powder, almost tasteless and insoluble in water. Repeated small doses are considered more toxic than occasional large ones. The dye is mostly excreted in urine which is tinged red 20-30 minutes after its administration; it has been shown to act as an urinary antiseptic. This dye is shown to be very suitable for children. The dosage advised by him is 8 gms. daily for at least 5 days for adults; 2 gm daily for 7 days for children under 8, and half this dose for children under 4.

Others recommend doses of from 15 to 30 gr. daily for a period of about 10 days. It is said to be effective, but it is not certain that it is safe. Its action appears to be cumulative, and it is capable of producing severe anaemia, presumably by a toxic action on the bone marrow.

The advantages claimed are that no dietetic restrictions are required and it does not seem necessary to use after purges.

Diphenan (Butolan). Diphenan is best known under the proprietary name of Butolan and is the carbamic acid ester of p-hydroxydiphenyl methane. It is probably the most effective anthelmintic for oral administration for thread worms. It does not produce serious toxic effects. The dose 0.5 gm three times a day for adults, and about half this for a school child. It is continued for a period of about two weeks and is very often successful.

Organic arsenicals in helminthic infestations. Some of the organic compounds of arsenic have been tried against intestinal parasites as well as in somatic infections. Given by the mouth stovarsol is said to have a vermicidal action. A 10 per cent solution of stovarsol has no effect on ascaris *in vitro* but when mixed with intestinal juices it becomes active. Injections of salvarsan have been used against clonorchis. Intravenously, organic arsenicals have been tried against oxyuris as well as guinea worm infections; against intestinal and blood flukes they have been tried without success. Neosalvarsan has been tried in hydatid disease in man without success. Experiments on rabbits show that hydatid material given with salvarsan does not prevent cyst formation.

Aniline dyes in helminthic infestations. The use of aniline dyes as anthelmintics is yet in its infancy. Methylene blue has been tried against

clonorchis in Japan with some degree of success. *In vitro* experiments show that living flukes are susceptible to methyl violet, crystal violet and nile blue sulphate. Methyl violet given intravenously to dogs stains the liver intensely and is excreted in the bile, but concentrations fatal to the worms are very toxic to the host; nile blue is even more toxic. Mercurochrome 220 (hydroxymercuric dibromofluorescein) and gentian violet have also been tried in clonorchiasis. The certified gentian violet (either penta-methyl or hexamethyl pararosaniline or else a mixture of the two compounds) is definitely toxic to the worms, and after doses of 50 mgm per kilo of body weight, by the mouth, produces a definite decrease in the egg count; smaller doses such as 16 mgm per kilo. are less effective. With 50 mgm. per kilo. however, symptoms of intoxication characterised by vomiting and loss of weight are produced; 35 mgm per kilo. produce no toxic effects, but in heavy infections where there is much damage of the liver, 15 to 17 mgm. per kilo. is a suitable dose. In man the results are not so good. Twenty ccm of a 1 per cent solution of gentian violet given intravenously, followed 3 days later by 30 ccm caused the disappearance of ova in one case.

Among other dyes, malachite green, brilliant green, crystal violet, fuchsine, congo red, trypan red, trypan blue and thionin blue have been tried. Both acid and alkaline dyes showed some toxicity on the cysticercus and on the larvæ, but no experiments have been carried out *in vivo*.

Hetrazan—This is a synthetic piperazine derivative (1-diethylcarbamyl -4-methyl-piperazine hydrochloride). Administered orally it produces marked and immediate (within 48 hours) reduction in microfilarial counts in the peripheral blood of cotton rats and dogs, infested with filarid worms. The continued administration of the compound to cotton rats at doses of 10 to 25 mg per kg orally, 3 times daily for 2 to 4 weeks, kills a large proportion of the adult worms. The exact mechanism of action is not known but it appears that it is different from antimony, arsenic and cyanine derivatives which have no such immediate and remarkable lethal effects on microfilariae. The acute toxicity of these drugs is low (mice—$L.D_{50}$—285 mg per kg given intraperitoneally, and 660 mg per kg given orally; rat—$L.D_{50}$—465 mg per kg given intraperitoneally and 1380 mg per kg given orally).

It has been used in human filariasis (*Wuchereria bancrofti* infection) in doses of 2 mg per kilo body weight three times a day being orally given after food for a period of three weeks. But in chronic cases this period may have to be extended. Smaller doses are not advisable since it has been shown that recurrences after small doses are more frequent which is an indication that all the filariae have not been killed. An average man weighing between 150 and 160 lbs would require 140 mg of hetrazan three times daily, *i.e.* 3 tablets (50 mg each) after each meal. The microfilariae are greatly reduced in number by the second day of treatment. Apart from action on the embryos, evidence of its action on the parent worms is suggested by the occurances of nodular swellings in some patients as a result of treatment and it is presumed that these nodules are dead or dying ones. The failure of microfilariae to reappear at all, or their appearance in insignificant numbers in the majority of cases, from 3 to 8 months after cessation of treatment, is indicative that some or all of the adult worms have been killed or have been made permanently sterile. Additional confirmatory evidence of effects produced against the adult worms is supplied by the symptomatology during treatment Dr. Michael Kenney states that "when clinical improvement is obtained it is always (with rare exceptions) following acute

exacerbation of usual filarial symptoms or sometimes other manifestations, which we consider of allergic origin and which must be produced by an increased amount of released filarial protein when the worm dies.

Druing treatment anorexia, slight nausea, malaise, weakness, fever, shortness of breath, etc. are sometimes seen but these usually pass off very soon. In some cases nodular swelling, testicular pains with temperature, etc. occur which are believed to be due to the death of the adult worms. Hewitt has reported evidence of toxicity in man at dosage of 8 to 10 mg per kg given after a meal, in volunteers not infested with filariae; and with a single oral dose of hetrazan ranging from 6.8 to 10.9 mg per kg after a noon meal the following major symptoms develop: malaise, nausea, drowsiness, lethargy, vasoconstriction, apprehension, increased salivation and perspiration.

This drug has also been used in *Onchocerca volvulus* infection in the same dose as in *W. bancrofti* infection and is found to be very highly effective. Extensive testing of hetrazan in Mexico and Guatemala, where about 1/3rd of the population are infested with Onchocerca, has shown that this drug has a highly specific and rapid effect upon onchocerciasis. Side reactions following its use for the treatment of this infestation have been fairly frequently reported but are of short duration, and they are not commonly a signal for discontinuance of treatment. The most convenient dosage is 2 mg per kilo body weight three times daily for 21 days. Precautions must be taken in administering this drug to patients suffering from onchocerciasis, since severe reactions after a single dose may occur, dependent apparently upon the intensity of infestation. Facial œdema, pruritis, and itching of the eyes are often encountered.

Treatment of chronic and advanced cases: No definite claims are made as yet for the usefulness of hetrazan in advanced clinical and elephantoid cases of bancroftian filariasis. Preliminary reports from British Guiana, indicate that subjective symptoms may be relieved, and that enlarged glands may be reduced in size after treatment. Treatment for a minimum of at least 4 weeks is advised in advanced clinical cases.

Effect of hetrazan against other parasitic round worms of man and domestic animals: It is effective however against several species of round worms other than filarids in both humans and domestic animals; thus in *ancylostoma braziliense* in man it is apparently effective in 2 mg per kg doses thrice daily for four weeks, and in—*Ascaris lumbricoides* in man, good effect is found at 2 mg per kg doses thrice daily for one day. In *Dirofilaria immitis* in dog good effect is observed against microfilaria and partial effect against adults at 25 mg per kg doses thrice daily for four weeks. In *Loa loa* in man, it is effective in 2 mg per kg doses thrice daily for 4 weeks. *In Acanthocheilonema perstans* in man, *Ancylostoma caninum* in dog, *Ascaris lumbricoides* in pig, *Dracunculus medinensis* in man, *Enterobius vermicularis* in man, *Mansonella ozzardi* in man and in *Necator Americans* in man, no definite data of its effect has yet been received. The diversity of activity exhibited by hetrazan in the nematode group of worms and its marked specificity for this group of worm infestation only is unparalleled.

The advantages of this drug for clinical use are: (*a*) toxicity is low. (*b*) specificity of the compound is very high for *Wvchereria bancrofti* and *Onchocerca volvulus,* (*c*) ease of administration is provided in that it may be given orally after meals, (*d*) stability of this drug is of advantage where it is to be used under varied conditions of climate and moisture. Hetrazan (Lederle)—Dose: 0.5 to 2 mg per kg body weight three times daily for 3 to 22 days.

PRESENT POSITION OF ANTHELMINTIC DRUG THERAPY

Although a large number of anthelmintic drugs have been introduced during recent years, only a small group of them are really effective without undue toxicity.

1. *Anthelmintic drugs acting on tissue parasites*:-

There are only a few drugs which have any marked degree of effectiveness on tissue helminths. Antimony compounds have a powerful action in schistosomiasis. Antimony tartrates of sodium and potassium are used; the former is considered to be less toxic. These drugs are used in the same way as in Kala-azar in 2 per cent solutions. The average dose for an adult is 2 ccm given intravenously, gradually increased to 5 ccm; 15 to 20 injections or more may be required to eradicate the disease. A preparation named fouadin or stibophen (sodium salt of antimony bispyrocatechol sodium disulphonate) is less toxic and has the advantage that it can be given intramuscularly. The first dose is 1.5 ccm, the second 3.5 ccm and subsequently 5 ccm. A total of 40 to 60 ccm are required. Following upon healing in a week or two the course may have to be repeated and thereafter the drug is given once a week and then every fortnight to prevent relapses. The drug is sold in ampoules containing 3.5 and 5 ccm (each 1 ccm containing 0.064 gm of fouadin). Emetine has also been used by intramuscular and intravenous routes for eradication of some of these infections but this drug is too toxic; gentian violet medicinal has been used by the mouth and intravenously but is still in experimental stage.

II. *Intestinal parasites*:—

Instructions for use.

For the treatment of intestinal worms, however, there are some quite effective remedies. General instructions for use of these drugs are:—(1) A definite diagnosis should always be made first based on demonstration of ova, larvae or segments of the parasite before treatment with these drugs is started. (2) All anthelmintic drugs are toxic and should be used with the greatest of care especially when pregnancy, debility, disease of the lung, kidney, liver, infectious fever are coexisting conditions. (3) Alcohol and fats favour absorption of some of these drugs and toxic effects are increased. These should, therefore, be forbidden. (4) A saline purgative especially magnesium sulphate is best used with these drugs. Purgatives, however, are contraindicated in inflammatory conditions of the gut (appendicitis, etc). If constipation is present bowels should be evacuated with an enema. Magnesium sulphate should be given on the preceeding night and again in the morning 2 or 3 hours after the anthelmintic drug has been administered. If, however, vomiting occurs and toxic symptoms appear it should be given at once. Many of these drugs act best when given on an empty stomach. Food should not be given till the bowels evacuate. Exceptions to this rule are hexylresorcinol in which food is witheld only for four hours after the administration of the drug and gentian violet which needs no purgative. (5) The drug selected should be one which is most effective in eradicating the particular parasite, has least toxicity and causes least inconvenience to the patient.

Aspidium.

Of the older drugs oleoresin aspidium even now is one of the best available for removal of tapeworms. The drug is especially dangerous in children with dwarf tapeworm infection and in such cases hexylresorcinol is preferred. Male fern should be given after proper preparation for two days before administration; the oleoresin is given in doses of 20 minims (1.25 ccm) in 3 doses in

capsules at half hours' interval in the morning on empty stomach followed two hours after by a saline purgative. Food is permitted after the bowels have been thoroughly evacuated (after one or two copious motions). Oleoresin can also be given in form of an emulsion, oleoresin 1½ dram (or 6 ccm), powdered gum acacia 2 drams (8.0 gm) and water two ounces (60 ccm). This is divided into two portions and given at interval of half an hour. In children one dram or 4 ccm of the emulsion is given for every ten pounds of body weight.

Recently carbon tetrachlorid in doses of 3 cc shaken with one ounce of saturated solution of magnesium sulphate has been used in tapeworm infection and found to be effective. Owing to the unstable nature of oleoresin aspidium and toxicity carbon tetrachloride is preferable.

Tetrachlorethylene.

Tetrachlorethylene is the best drug in hookworm infection when ascaris infection does not exist at the same time. The drug is given in gelatine capsules each containing 8 min (0.5 ccm) and to children it may be given on sugar. In mass treatment it is best given in a saturated solution of magnesium sulphate. Dose for adult is 45 min (3 ccm), for 10 to 15 years 30 min (2 ccm); children under that age 10 to 30 min (0.5 to 2 ccm) according to age. The patients should avoid fat in diet 24 hours previously; no alcoholic beverages are allowed. The night before treatment a saline purgative (not castor oil) is taken; only light liquid diet is allowed. In the morning following, the drug is taken on an empty stomach followed one hour after by a dose of magnesium sulphate. The patient should lie down and rest till the bowels are evacuated. Then fruit juice with plenty of sugar is given; fatty food and alcoholic drink should be avoided for next 24 hours. The same treatment has been found to be effective in *T. Solium* and T. Saginata infestations.

Hexylresorcinol.

Hexylresorcinol is the drug of choice for ascaris infection and especially when hookworm infection is also present. It has some value in tapeworm infections also. Hexylresorcinol has been comparatively recently introduced and is as near an ideal anthelmintic as possible. Little or no toxic symptoms are produced and contraindications are few. It can be given while the patient is doing his ordinary work and there is no danger of cumulative toxic effects. Its effectiveness against ascaris is no less than that of santonin or chenopodium and even against hookworm the only drug which excel it is tetrachlorethylene. Two doses of the drug at interval of two weeks suffice to clear round worms as also many hookworms. It compares favourably in the latter infection to a combination of tetrachlorethlyene and chenopodium, especially when ascaris infection is co-existing. The second dose is only needed when the first has not entirely cleared the worms.

Pills of hexylresorcinol should be put right back on the tongue and swallowed; they should not be chewed as they irritate the mouth. The drug should always be given on an empty stomach otherwise it loses effectiveness. A preliminary saline purgative essential but the post-purgative is not essential except to clear out the dead worms 24 hours after. Hexylresorcinol crystoids are the convenient form for administration. The dosage recommended in children is 0.1 gm per year of age up to 10 years and above that 1.0 gm.

Hexylresorcinol is also effective against *Fasciolopsis buski*, whipworms, tapeworms and pinworms infections. In tapeworm infection repeated doses are said to be more effective and generally three doses have to be given on alternate mornings with magnesium sulphate the night before. The diet should be light and fruit juices with plenty of sugar should be given.

In pinworm (enterobius) infection the "crystoids" are used in usual doses with a high enema of its solution (1—2,000) which is retained for 20 minutes if possible. To avoid irritation the anal region should be thoroughly washed and dried. The treatment can be repeated after every two weeks.

Gentian Violet.

Gentian violet is still in experimental stage, but from the results so far obtained, it is simpler to administer and more effective than hexylresorcinol in oxyuris infections. It is said to be specific in the treatment of strongyloides and *Clonorchis sinensis* infections. Tablets of gentian violet are prepared, enteric-coated, but these may pass undisolved through the gut. Tablets of ¼ and 1/2 grain each dissolving after 1, 1½ and 4 hours are available. In children weighing less than 100 pounds, ½ gr (0.0325 gm) is given three times a day for fourteen days one hour before meals. The treatment is discontinued for seven days and the above course is repeated. Adults weighing more than 100 pounds receive 1 gr (0.06 gm) three times a day one hour before meals for seven days, rest for seven days and then the course is repeated. The cure rate with this treatment is 90 per cent in pinworm (oxyuris) infections.

In clonorchis and strongyloides three ½ grains tablets per day are taken in divided doses till 50 grains are taken.

Other Anthelmentic Drugs.

Santonin is a time honoured anthelmintic against ascaris infection but is now considered to be not a very effective drug; besides it is toxic.

Leche de higueron is the crude sap of a bastard fig tree in Central and South America and is effective in the treatment of trichuris infections. There is no method of preserving this drug so that it is only of local use.

PART III

REMEDIES USED AGAINST PROTOZOAL DISEASES

PROTOZOAL DISEASES

INTRODUCTORY AND GENERAL—CLASSIFICATION OF PROTOZOA INFESTING MAN AND ANIMALS; VACCINE THERAPY IN PROTOZOAL DISEASES—DYSENTERIC DISORDERS: AMOEBIASIS (INTESTINAL); CLINICAL ASPECTS; THE PROBLEM OF CURE; CHEMOPROPHYLAXIS—SECONDARY AMOEBIASIS: HEPATITIS AND HEPATIC ABSCESS; RARE AMOEBIC LESIONS—IPECACUANHA DERIVATIVES; EMETINE: PHARMACOLOGICAL ACTION, EMETINE IN THERAPY, DOSAGE AND MODES OF ADMINISTRATION; EMETINE BISMUTH IODIDE; MODES OF ACTION AND TOXIC EFFECTS OF EMETINE—QUINOLINE DERIVATIVES: CHINIOFONUM, DIODOQUIN, VIOFORM AND ENTEROVIOFORM—ARSENICAL COMPOUNDS—HOLARRHENA ANTIDYSENTERICA—OTHER DRUGS IN AMOEBIASIS—SUMMARY OF TREATMENT OF AMOEBIASIS—PALLIATIVE REMEDIES IN AMOEBIASIS: PLANTAGO OVATA (ISPAGHULA), AEGLE MARMELOS (BAEL FRUIT), ACORUS CALAMUS.

1. Introductory and General

Protozoa are defined as non-cellular animals, *i. e.*, the body of the individual animal is not subdivided into cells. The body substance is composed of cytoplasm which can be differentiated into *ectoplasm* and *endoplasm*. The ectoplasm forms the organelles of locomotion such as pseudopodia and flagella and forms a protective covering to endoplasm. The nucleus lies in the endoplasm. Reproduction may be (1) sexual which is usually completed in two different hosts and (2) asexual in one host.

The protozoal organisms attack animals, multiply in their bodies and produce a train of symptoms which differ with different organisms. The following table according to Stitt gives the systematic position of the protozoa which infest man:—

(1) Classification of Protozoa Infesting Man and Animals

Class	Order	Genus	Species
I. Sarcodina (Rhizopoda) Move usually by means of protoplasmic projections called pseudopodia and multiply by binary fissure in the active stage and by encystation.	Gymnamoebida	Endamoeba	E. histolytica E. coli E. gingivalis
		Endolimax	E. nana
		Iodamoeba	I. butschlii
		Dientamoeba	D. fragilis
II. Flagellata (Mastigophora) Move by mans of undulating membranes or flagellum, multiply by division of the body longitudinally into two.	Protomonadida	Trypanosoma	T. gambiense T. rhodesiense T. cruzi
	Monozoa	Leishmania	L. donovani L. infantum L. braziliensis L. tropica
		Trichomonus	T. hominis T. vaginalis
		Chilomastix	C. mesnili
		Embadomonas	E. intestinalis
		Enteromonas	E. hominis
	Diplozoa	Giardia	G. lamblia

(1) *Classification of Protozoa—(Contd.)*

Class	Order	Genus	Species
III. Infusoria (Ciliata) Move by means of numerous fine cilia and glide about swiftly, multiply by transverse division of the body into two and also produce resistant cysts.	Heterotrichida	Balantidium	B. coli
		Nyctotherus	N. faba
IV. Sporozoa These have no motor organs; they live parasitically in the cells or tissues of other animals. Reproduction by spores.	Coccidiida	Eimeria	E. stiedae
		Isospora	I. hominis
	Haemosporidia	Plasmodium	P. vivax
			P. malariae
			P. falciparum
			P. ovale
			P. knowlesi
	Sarcosporidia	Sarcocystis	S. tenella

The commonest diseases which are caused by protozoal organisms in tropical climates may be grouped as follows:—

(1) Amœbiasis (dysentery, etc.).
(2) Leishmaniasis (kala-azar).
(3) Trypanosomiasis (sleeping sickness).
(4) Hæmosporidia infections (malaria).

Specific drugs.

From very early times, attempts have been made to influence these diseases by drugs. Countless drugs were introduced empirically from time to time for the treatment of parasitic diseases only to be discarded after more prolonged trials. The work of Ehrlich opened up a new field for the scientific assessment of the value of drugs. During the years that followed, rapid advances were made in this field of chemotherapeutic research. Helminths, protozoa, and spirochætes can be readily detected by direct examination by the microscope and hence the influence of drugs on these organisms has been studied in an elaborate fashion. Though the mode of action of these drugs has not been clearly explained, certain interesting data have been obtained. Emetine and its derivatives and the cinchona alkaloids have been found to act on some protozoal organisms, while compounds of arsenic, antimony, bismuth, and aniline derivatives have a well-marked toxic action on others.

The recognition of trypanosoma infections in experimental animals and their behaviour under the influence of drugs injected or administered otherwise, has made possible the study of the mode of action of various remedies under laboratory conditions. It has been found that different drugs have different degrees of lethal action on the parasites. It has also been shown that although, in a general way, there is relationship between action of drugs *in vitro* and their activity *in vivo,* yet there are many exceptions. Drugs appearing strongly toxic to an organism *in vitro* may have very little or no action at all against the organism *in vivo.*

During recent years, certain definite advances have been made in the chemotherapeutic treatment of protozoal diseases.

Classification of Drugs Acting on Protozoal Infections

(1) *Amoebiasis* and intestinal flagellate infections. Ipecacuanha and its derivatives; Quinoline derivatives such as chiniofon, diodoquin and vioform;

organic arsenicals such as carbarsone and stovarsol. Holarrhena antidysenterica and its derivatives, etc. Such drugs as *Plantago ovata* (Isabgul), *Aegle marmelos* (Bael fruit) have only palliative action.

(2) *Leishmaniasis.* Antimony compounds, arsenicals, Bayer 205, diamidino stilbeine, etc.

(3) *Trypanosomiasis.* Arsenicals; Bayer 205 and Fourneau 309 (Moranyl); antimonials, bismuth compounds, quinoline derivatives, guanidine derivatives, etc.

(4) *Haemosporidial infections.* (Malaria). Cinchona alkaloids, plasmochin, mepacrine, paludrine, etc.

(2) Vaccine Therapy in Protozoal Diseases

Immunity in protozoal diseases.

During recent years evidence has been brought forward to show that immunity may develop in protozoal diseases, and in accordance with this hypothesis vaccines have been tried by several workers in the treatment of protozoal infections. It must be pointed out, however, that in contradiction to bacterial diseases, the phenomenon of immunity in protozoal infections generally is still problematical and vaccine therapy in the latter group is yet in experimental stage.

For the rational and successful employment of vaccine therapy in infectious diseases in general, three conditions have to be satisfied: (1) The ætiological agent of the disease should be one that can be isolated and grown in a form suitable for the manufacture of vaccine; (2) the mechanism of cure of the disease should be one in which the natural processes of defence such as the antibody and the phagocytic mechanisms play an important part; (3) the treatment of the disease with drugs should be in an unsatisfactory state and sufficient experimental evidence present to indicate that vaccine therapy will be beneficial when employed alone or in combination with drug therapy. Applying these three criteria to vaccine therapy in protozoal diseases we find that there is very little need or justification at present for the employment of this type of therapy in human beings except in a few conditions in which specific drugs of value have not yet been discovered. The following is a brief outline of the evidence on which the above conclusion is based.

Difficulties of growing protozoa in vitro.

Judged from the point of view of the first criterion mentioned above we find that there are many technical difficulties in the way of preparing suitable vaccines out of most of the pathogenic protozoa that infect man. Protozoal organisms compared to bacteria, are rather difficult to grow in artificial culture media and the only protozoa that have so far been successfully cultivated on a large scale and in a form suitable for vaccine production are *Leishmania tropica, Leishmania donovani,* and *Entamoeba histolytica.* Others such as the trypanosomes, plasmodia, etc., are not only difficult to grow outside the body but it is also not easy to obtain them in a pure state for vaccine manufacture. The types of protozoal vaccine that have commonly been employed in experimental work on laboratory animals are (*a*) attenuated living organisms, (*b*) virulent organisms which after inoculation are controlled by treatment and (*c*) dead or disintegrated organisms and their products. For use in human beings, the first two are unsuited and the third which may prove useful is not available in all cases.

Role of antibodies.

Judged from the point of view of the second criterion, vaccine therapy does not appear to be any more satisfactory. The chief object in injecting vaccines being the elaboration of antibodies for the purpose of destroying the infecting agent, we would naturally wish to ascertain, before employing it, whether (*a*) there is sufficient evidence to prove that antibodies are formed in protozoal infections, and (b) if so, whether these antibodies play any important part in overcoming these infections. Although our knowledge of the immunity mechanism in protozoal infections is still imperfect, there is evidence that antibodies are formed in certain protozoal diseases; but in these diseases there is no certainty that the antibodies elaborated are of real value in immunity. In trypanosomiasis, the lytic substances that are formed are supposed to play a part in the overcoming of these infections; but, both on account of the complexity of the antigens present in these organisms as well as due to their possessing powers of adaptation and resistance against the injurious action of antibodies it is not possible to assess correctly the value of antibodies in immunity to protozoal diseases. Furthermore there is evidence to suggest that in other protozoal infections as for example the plasmodium infections, immunity is dependent upon the

sensitiveness of the phagocytic mechanism and also upon certain non-specific factors such as alterations in cell permeability, variations in bio-chemical conditions and in the nature of available food supply. If it is so then it is still more difficult to say how far the use of specific antigens (vaccines) and the presence of specific antibodies can be of benefit in overcoming these infections.

Judged from the third and last criterion, we find again that vaccine therapy in protozoal diseases can at best be only of limited value. The treatment of most of these diseases with drugs is in quite a satisfactory state and there is little need for resorting to vaccines to bring about a cure. In malaria, visceral leishmaniasis, amœbiasis, and trypanosomiasis, specific drugs of proved merit are readily available and no practitioner would ever dream of employing vaccines in preference to them.

Taking the evidence discussed above as a whole, the manufacture and use of vaccines so far as the treatment of most protozoal diseases is concerned, may justifiably be said to be a matter of pure research or academic interest. The results of practical employment of vaccines in the different protozoal diseases recorded below further substantiate this view.

Immunity in protozoal diseases.

Oriental sore. It was noticed long ago that recovery from an attack of oriental sore conferred immunity to a second attack. This observation was put to practical use and individuals were inoculated with material from a sore, but results were not encouraging. Row (1912) tried the effect of vaccines in the treatment of cases of oriental sore and claimed that he obtained favourable results. Ray employed a vaccine prepared in a different way to Row's (organisms were grown on solid media and killed by freezing and thawing) and claimed that it possessed remarkable curative properties. His claim has not yet been corroborated by others.

Kala-azar. One attack of kala-azar protected dogs and monkeys from another attack. This led to attempts to treat kala-azar in man with vaccine but the results obtained were unsatisfactory.

Dermal leishmaniasis. A vaccine was employed in dermal leishmaniasis but the results have been very variable. While in a certain number of selected cases a small degree of temporary improvement was noticed, in the majority of unselected cases little benefit was obtained.

Trypanosomiasis. The usefulness of vaccine in trypanosomiasis has been tested only in laboratory animals. Serological tests conducted after the use of vaccines have revealed specific changes in the blood serum and complement fixation tests have yielded positive results.

Plasmodium infections. In the case of plasmodial infections birds it has been said that inoculations into canaries, of sporozoites which have been rendered non-infective by keeping them for 12 to 48 hours after removal from mosquitoes, rendered them immune to inoculation with virulent parasites. In human beings on the other hand the immunity to reinoculation that develops in the course of malarial therapy, does protect these individuals from infection when sporozoites from mosquitoes are injected.

Piroplasmosis. Vaccination with the living organism followed by treatment with specific drugs has been successful in prophylaxis.

Babesia infections. Using a vaccine made from macerated spleen and lymphatic glands of animals infected with babesia, it has been claimed that about 50% of the animals develop immunity and are resistant to re-inoculation.

Conclusion.

From the above discussion it will be evident that the use of protozoal vaccines has so far yielded interesting results from an immunological point of view in laboratory animals but their employment in man as a therapeutic agent has furnished neither successful nor encouraging results. It may be possible that with improvements in the technique of preparation, standardisation and administration of protozoal vaccines we may obtain in future more encouraging and beneficial

results; but at the present time their value seems to be doubtful. Neither vaccines nor sera have any place in the treatment of these diseases at present.

2. Dysenteric Disorders

The term 'dysentery' is derived from Greek word meaning 'complaint of the bowel'. According to popular idea, it means passage of blood and blood stained mucus in the stools, generally accompanied by abdominal discomfort or pain, straining and tenesmus. The disease is of wide etiology but the symptoms of various groups may resemble each other in many ways. There are three main groups of dysentery:—

I. *Bacillary or epidemic dysentery* produced by the dysentery group of bacteria, *e.g.*, Shiga, Flexner, Sonne etc. These will be discussed under Bacterial Diseases.

II. *Protozoal dysentery*

III. *Metazoal or Helminthic dysenteries* (*Bilharzial* and *Verminous*) discussed under Helminthic disease.

Protozoal or Endemic Dysentery

Manson-Bahr has classified Protozoal dysentery as follows:—

(*a*) *Amoebiasis* or infection with *Entamoeba histolytica* (Schaudinn). This term includes:—

1. Primary intestinal amœbiasis or amœbic dysentery.
2. Secondary amœbiasis, *i. e.*, complications, such as hepatic amœbiasis, amœbic liver abscess, etc.

(*b*) *Balantidiasis* or infection of the intestines with an infusorium *Balantidium coli.* This is rare in man though common in animals.

(*c*) *Giardiasis* or *lambliasis,* infection of the bowel with *Giardia lamblia* a protozoal flagellate.

(*d*) Flagellate diarrhoea or infection with *Trichomonas hominis* and *Chilomastix mesneli* which is rather a doubtful entity.

(*e*) *Coccidiosis* infection with *Isospora hominis,* a rare infection of the gut in man.

(*f*) *Malarial dysentery* occuring in the course of M. T. infection(*P. falciparum*).

(*g*) *Leishmanial* dysentery occuring in the course of kala-azar.

Amœbiasis or entamœbiasis includes tropical, endemic or amœbic dysentery or intestinal amœbiasis. Its secondary manifestations are amœbic hepatitis, and amœbic abscess of liver, lung, brain, spleen and epididymus. Amœbic ulceration of skin also occurs. The aim of the parasite is to infect man without causing death of the host.

CHAPTER I

AMŒBIASIS

(1) Amœbiasis (Intestinal)

A number of amœbae infest man and animals but they do not all produce disease. All authorities now consider that *E. histolytica* is the pathogenic organism. The pathogenicity of *Entamoeba histolytica* and the harmlessness of *E. Coli* in man have been demonstrated. The two organisms look very much alike, but they can be distinguished by the fact that in the protoplasm of *E. histolytica* numerous red corpuscles are present, while *E. coli* contains bacteria and other foreign bodies. The cystic stage of the parasitic organism has a thin wall and usually contains four nuclei which can be stained with double strength iodine solution, while fully developed *E. coli* cysts have eight nuclei. The cysts appear in the stool when the acute dysenteric symptoms have subsided and they constitute the resistant stage. The cysts can live outside the body for upto three weeks and they cause new infections when swallowed with food. Encystation can be produced in cultures of *E. histolytica* under certain conditions. Experiments show that the cysts do not develop in the small intestine but in the upper part of the large intestine where plenty of fluid is present. Dobell (1928) described the process of excystation with the escape of a single quadrinucleate amœbae through a minute perforation in the cyst wall. No sexual phenomena have been observed during the metacystic stage.

Races of E. histolytica.

Craig has discussed the possibility of existence of a completely non-pathogenic strain of *E. histolytica* (*E. dispar* of Brumpt) and the question of existence of races of *E. histolytica* of varying virulence and the subject of immunity after amœbic infection. The answer to the first question is probably negative and to the second is in the affirmative, while the answer to the third cannot be given as the evidence is insufficient inspite of the knowledge that specific complement-fixing bodies occur in the blood of animals and individuals infected with *E. histolytica.* Long continued infection may lead to relative immunity.

Races of *E. histolytica* producing small cysts have been encountered. As a result of experiments designed to compare the small race with the ordinary one, it was found that the small race was more difficult to cultivate, and that, in the culture, the amœbae whether small or large, were reaching the size of the amœbae in cultures of the large race.

E. histolytica can be cultured without difficulty on a medium containing human serum, dextrose, NaCl, KCl and $NaHCO_3$. The culture from stools is of practical value in establishing the presence of the organism when microscopical examinations have failed. Hæmolytic, cytolytic and complement-binding substances have been obtained from the cultures by extraction with alcohol and a complement-fixation test for the diagnosis of *E. histolytica* infections has been worked out.

Non-pathogenic entamœbæ occur in the human intestine. *E. coli* is by far the commonest organism. *Endolimax nana* resembles a small *E. coli;* its oval cyst contains four nuclei. *Iodamoeba butschlii* has a vesicular nucleus and its cysts have a signet-ring-like nucleus with a large and very prominent glycogen vacuole. *Dientamoeba fragilis* shows two nuclei. *E. dispar* can only be differentiated from *E. histolytica* by injection into the rectum of kittens.

Normal habitat.

The normal habitat of *E. histolytica* is the mucous membrane or sub-mucous layers of the large intestine where it feeds on the cells and dissolves the tissus with the proteolytic ferment it produces. The organism multiplies without producing much detriment to health. Amœbic cysts are passed in the stools and in such persons there may not be much evidence of ill health. In these persons there are always some lesions in the intestines which may be very small, but which under certain circumstances may develop and produce signs of amœbic dysentery or amœbic affection of the liver.

In the intestines all degrees of ulceration may be present from the minimal producing no marked symptoms to passage of blood and mucus, with active amœbae. The symptoms are much milder than those in bacillary dysentery and most of the individuals suffering from amœbic infection are more or less healthy. They are, however, a source of infection to others as they pass the infective organisms in their fæces. In only a small proportion, are the liver and other organs affected.

Mode of transmission.

Infection with *E. histolytica* is brought about by ingestion of cysts; fæcal contamination of food and water being mostly responsible. Cysts can survive and remain infective in water and fæces for several weeks. All unprotected wells, open tanks and rivers may be infected. Infection is less common in cities with proper water supplies.

The cysts when ingested pass through the stomach and excyst in the small intestines; a metacystic amœba with four nuclei emerges from each cyst. After complex metacystic development eight small amœbulæ are produced from each metacystic amœba. The amœbulæ grow and divide by biniary fission into active trophozoites which invade tissues and produce disease in the large intestines. When required conditions develop, cyst formation occurs and the cycle is repeated.

Amœbic infection unlike bacillary dysentery, does not usually occur in epidemics but in form of sporadic cases. Occurence of a large number of cases often means a common source of infection. Under tropical and sub-tropical conditions, the house fly is the common disseminator of infection. The disease is also transmitted by water. Parasitic cysts may be ingested with green vegetables and raw fruit where human 'night soil' is used as manure. Amœbic cysts can survive in fæcal contamination of the fingers for 45 minutes; in dry fæces at room temperature they die very quickly. Lowered resistance of the patient to disease is an important factor in the incidence of the disease. The disease occurs at all ages but is comparatively less frequent in children; men are more commonly affected than women. Amœbiasis does not exhibit that seasonal incidence which is characteristic of bacillary dysentery, though the risk of fæcal contamination is greater in rainy season.

Cyst-Carriers.

There are 'healthy' cyst carriers who are appareantly normal. Such persons harbour living entamœbae in their tissues. There are also convalescent carriers. The lesions of the mucosa may be very minute and may be only visible in microscopic sections of the bowel. The carrier has been defined as an individual in whom *E. histolytica* is living as a *commensal.* In all carriers superficial necrosis of intestinal epithelium continually occurs over microscopic areas and this is often followed by rapid regeneration and healing but there may be definite ulceration. The incidence of cyst-passers in a community is an index of the prevalence of amœbic dysentery. It has been pointed out that incidence of intestinal infection with *E. coli* affords some indication of the probable rate of infection with *E. histolytica;* indeed it seems that infection with *E. histolytica* is proportionate to the degree of infection with intestinal protozoa in general.

Distribution.

Although the disease is dependent more on defective sanitary conditions, there is reason to believe that amœbiasis is more prevalent in tropical and sub-tropical countries than in temperate and colder regions. Amœbic cyst carriers occur in Northern countries, but the incidence of amœbic dysentery and liver abscess are very low. As the Southern countries are approached amœbiasis becomes increasingly more common. Indigenous amœbic dysentery is present in England and France and most countries of Europe and America. In India amœbiasis is widespread especially in the provinces of Bengal and Bombay. A survey made by the School of the Tropical Medicine, Calcutta, showed an incidence of 12 per cent among the population but this figure is probably on the low side.

Pathology.

Mature living cysts of *E. histolytic* when swallowed pass through the stomach unchanged. In the small intestines the wall of the cyst is absorbed and the young amœbae pass on with the intestinal contents into the large intestines where they establish themselves. Amœbic dysentery is a disease of the large intestines, but occassionally it attacks the small intestines, the symptoms then resembling those of a bacillary infection.

The young amœbae first attack and destroy the columnar epithelium and then pass down the crypts of *Lieberkühn ;* they penetrate the basement membrane and reach the submucosa. Here they attack the tissues by means of the powerful cytolytic ferment which they secrete. By active proliferation, nests of amœbae form which multiply rapidly with the rich nourishment they obtain from the tissues. The amœbae engulf red corpuscles leucocytes and remnants of tissue cells. The earliest lesions are yellow papules or elevation containing pus which open by sinus formation into the submucosa. The initial ulcers formed in the mucous membrane as seen by sigmoidoscopy are small punctiform depressions. Abscesses in the submucosa tend to spread and burst into the lumen producing ulcers with a narrow neck, broad base and undermined edges, which on vertical section assume a flask-like shape or a button hole appearance. Deep ulcers have swollen or raised edges and are surrounded by numerous hæmorrhages. The smallest ulcers look like granular patches on the mucous membrane and can only be seen with the aid of a magnifying glass. The mucous membrane between the ulcers is normal in colour and texture. Solitary ulcers are seen as low down as the rectum, with long hairy black sloughs frequently projecting into the lumen. The whole surface of the mucosa may be covered with a stringy

black exudate looking like sea-weed. The walls of the caecum and ascending colon may become attenuated and dilated, the portion between the ulcers may buldge like small balloons. In chronic cases the wall is thickened and fibrosed and its physiological functions are interfered with. Shallow ulcers with undermined edges and brownish bases formed of granulation tissue are of bacillary origin and the infecting organism can be isolated from these. Healed amœbic ulcers take the form of smooth, depressed, non-pigmented scars, while those of bacillary origin are pigmented and very friable.

The character of the stool depends upon the size and nature of lesions. Stools are offensive when there is a lot of necrotic tissue. The lesions are most extensive in the proximity of the flexures and in severe cases the mucosa of the whole of the large gut may be involved.

Experimental work on cats shows that *E. histolytica* first attack the large bowel, especially the lower part immediately above the anal ring. Amœbae enter the connective tissue or into the crypts or the lymphatic channels, from where they migrate into the lymph follicles and the submucosa. Ulcers found in the rectum are the smallest and can be recognised by sigmoidoscopy. Stasis is of importance in intestinal amœbiasis as at such sites there is a special tendency for the amœbae to invade the bowel. The appendix is affected in nearly half the cases and is usually involved when there are extensive lesions in the cæcum. Intestinal gangrene involving small portions or even the whole of transverse colon may occur through thrombosis of submucous vessels. Hæmorrhage and perforation (especially in the cæcum and appendix) are two of the accidents which may occur in intestinal amœbiasis. Localised peritoneal abscesses may be formed by walling off of the peritoneum in the neighbourhood of a perforated abscess. Post mortem, few signs of toxic absorption and few visceral changes are seen.

Microscopically the smaller capilliaries show stasis, then become thrombosed, with resulting œdema and coagulation necrosis. Amœbae invade the walls of the thrombosed vessels and can be seen in the coagulated blood; they probably gain entrance into the portal circulation from here and so reach the liver. Beyond the cellular infiltration, the lymphatics are dilated and their contents are coagulated.

Healing of amœbic ulcers occurs by separation of sloughs and appearance of granulation tissue on the floor of the ulcerated area; in this fibrous tissue, a scar of slate-grey colour is formed. Regeneration of the mucous membrane even in extensive ulceration is rapid and scars are quickly covered over with epithelum.

(2) Clinical Aspects

E. histolytica on the whole endeavours to live in harmony with the human host, but acute and rapidly fatal cases may be seen. As a rule the course of the disease is long drawn out and the patient though passing blood and mucus in the stools, may lead an active and useful life. The infection may simulate almost any gastro-intestinal condition. Although in acute amœbic dysentery emaciation may occur, in the chronic form this is rare. Death may result from perforation of ulcers or erosion of some large blood vessel in the intestinal wall. *Symptoms.*

In carriers, some variations from the normal must occur. The incubation period is of considerable length and in experimental infections in man was found to be on an average a little over two months. It ranges from 7 to 15 days but symptoms may occasionally appear in a week while in other cases they may take 3 to 4 months. The patients can be divided into the mild, the acute, the fulminating and the chronic cases, the last forming the largest group. Intestinal amœbiasis is one of the most persistent of infections and a person once infected heavily, remains so for life unless properly and intensively treated. The symptoms may vary from simple indigestion to those produced by new growths in the bowel. The onset is generally gradual and progressive. It often commences as mild diarrhœa which may last several days and then blood and mucus may appear in the stool. In a large group it may not go beyond the stage of amœbic diarrhœa and often there is alternating diarrhœa and constipation. Intense abdominal symptoms, such as those occuring in bacillary dysentery, are absent. There is, however, considerable abdominal discomfort. Fever is usually absent, but rarely hectic fever occurs due to septic absorption from the ulcers. In some epidemics, acute onset with fever, intense abdominal pain and tenesmus have occured and the condition has been mistaken for appendicitis.

The stools are more copious than in bacillary dysentery and seldom exceed twelve in 24 hours; usually there are only 3 or 4 which contains dark blood and mucous resembling 'anchovy sauce'.

Pain may be localised in some parts of the large intestines and is of a subjective nature. It is usually confined to the sigmoid and descending colon or to the area of caecum and appendix. The involvement of the cæcum may be mistaken for an acute attack of appendicitis. Pain elicited on deep pressure in the centre of the sigmoid flexure opposite the anterior superior spine of the ilium according to Manson-Bahr is of special diagnostic significance and might be termed 'the amœbic sign post'. Pain may be localised in the centre of the transverse colon and may be mistaken for some affection of the gall bladder. Pain may also be referred to the lumbar region, and may lead to a mistaken diagnosis of pyelitis or renal calculus.

The bowel symptoms, that is, diarrhœa with blood and mucus, are usually quite painless; in some cases the main symptom is constipation. Flatulance may persist and gaseous distension of colon and caecum is a cardinal sign of the disease. This may produce symptoms of acute dyspepsia and even those of duodenal ulceration.

Palpable abdominal tumours with blood stained discharge might suggest malignant disease.

Fulminating cases of amœbic dysentery are very rare and in these, massive destruction of intestinal mucosa may produce septicæmia and death.

Chronic amoebiasis.

Many cases with cysts in their stools do not show any gastro-intestinal symptoms; some have mild symptoms to which they become accustomed and therefore they are not aware of the disease. A large majority of cases of chronic amoebiasis met with in India belong to this category. They do not even give a history of ever having suffered from an attack of dysentery. They are in fact surprised when they are told that they have amœbiasis. They are a source of danger not only to themselves but also to the community in which they live.

Others suffer from attacks of slight diarrhœa or constipation, indigestion, distension of abdomen after meals, loss of appetite, lassitude, and dull aching pains in the back, extremities and lower part of the abdomen. The complexion is sallow and the face has a peculiar dull flabby and under nourished appearance. The patients may be nervous and irritable. Often there is mental depression. The pulse is generally of low tension, irritable and may be arrhythmic. On deep palpation there is tenderness of the large intestines and over the liver region. Large gut may be thickened and palpable.

Complications.

Perforation may be fatal. Hæmorrhage from a perforated artery may occur. Acute amœbic hepatitis occurs in about 5 per cent of cases. Sacculation of the large intestine with dilation may occur. Intestinal amœbiasis and sprue often co-exist. Cachexia may rarely occur. Prolapse of rectum is rarely seen. Pericolic abscess leading to partial or complete obstruction has been met with. External piles are often present. Spasticity of sigmoid colon is frequently seen in intestinal amœbiasis. Infection of ulcers with organisms, such as streptococcus *or B. coli* may produce secondary infections. Neurasthenic symptoms may be produced and these are often referred to as 'tropical neurasthenia'. Gastric and duodenal ulceration or diverticulitis may occur. The gastric secretion is not affected. Strictures of the bowel are very rare.

Diagnosis.

Microscopic examination of stools shows *E. histolytica* in motile and dividing or cystic forms, the former only being present when blood and mucus are being passed. Carriers of *E. histolytica* have normal stools with cysts but the large tissue invading forms which have ingested red blood cells are only rarely present. It is exceptional to find the tissue-invading forms in chronic amœbic dysentery. The organism is found in the blood streaked mucus and in the clots. Single amœbae or clumps may be demonstrated. The exudate may fail to reveal the parasite. In cases with secondary lesions, such as liver abscess, amœbæ are usually absent in the stool. In the chronic stage, cysts are difficult to find at times, but on other occasions they may occur in large numbers. Stools should, therefore, be examined for six or more consecutive days before a negative diagnosis is made; the

writer has found them after twenty negative examinations. Amœbæ and cysts usually appear after a saline purgative; castor oil should be avoided as the oily droplets make examination of stools difficult. The fæces should be as fresh as possible (passed immediately or not longer than one hour) especially in the acute stage as vegetative forms rapidly degenerate outside the body and die within two hours. The cysts, however, remain alive and recognisable in the stool for many days, sometime several weeks under ordinary conditions of moisture and temperature. In trained hands positive observations may be made in 75 per cent of cases on first examination and in 90 per cent after the second examination. Examination should be made on a warm stage. Charcot-Leyden crystals are present in 25 per cent of cases of intestinal amœbiasis but these are not pathognomonic as they occur in intestinal stasis of all forms. The sigmoidoscope may reveal lesions in the rectum in 80 per cent of cases. These lesions are quite distinctive and amœbæ can be demonstrated in scrapings from the ulcers. In the acute stage the examination with sigmoidoscope is painless as compared with bacillary dysentery. Ulcers are scattered and the intervening mucosa is normal; the individual ulcers are only a few mm in diameter and small petecheal hæmorrhages are frequently seen. The earliest amœbic lesion in the rectum is a small yellow elevation with a hyperaemic margin. The lesions may coalesce producing a ragged granulating and bleeding surface resembling bacillary dysentery or ulcerative colitis; they may extend down to the sphincter ani. Solitary amœbic ulcers up to 2 cm in diameter may occur. The chronic stage is difficult to diagnose by laboratory methods.

After an initial attack of amœbic dysentery the patient may remain free from any external signs and symptoms of disease from 5 to 10 years. The lesions in these cases take the form of yellow papules or the whole surface of the mucosa may be studded with microscopic pits or depressions representing healed ulceration. In the carrier stage the bowel is usually normal but sometimes granular patches or small scattered hæmorrhages may indicate minute amœbic lesions. Sigmoidoscopic examination may afford an excellent guide to treatment and rapid healing of lesions may be observed under treatment.

There is moderate leucocytosis, about 10,000 per cmm, and cases showing a higher count may possibly be liver abscess. Leucocyte count is generally higher in intestinal amœbiasis than in bacillary dysentery. Complement fixation test and intradermal tests are not of very great help and X-ray appearances with opaque meal are not suggestive.

Errors in laboratory diagnosis.

The value of the culture method as a means of diagnosis of *Entamoeba histolytica* infection was examined. It has been found that in culture, *E. histolytica* and *coli* may come to resemble one another so closely that their differentiation in the tissue in which the diagnosis has to be made may be quite impossible. The culture method for diagnostic purposes may then be misleading. Reliance has to be placed on examination of fresh and fixed fœcal specimens which will give an accurate diagnosis only when typical free or encysted *E. histolytica* are present. Necessity of freshness of stools cannot be too strongly emphasised and it is advisable that stool be passed in the laboratory if possible. It is a mere waste of time to examine stale stools. Fæces obtained after the ingestion of oil, bismuth or arsenic or after a saline purge are liable to lead to errors.

(For details see Part II)

(3) The Problem of Cure

Dysentery is a clinical syndrome implying the passage of blood, pus and mucus with the stool accompanied by tenesmus, and the term has been loosely used even to cover the carriers where these symptoms are absent. Some use the word 'cure' in the sense that vegetative and cystic entamœbæ are absent while others use it to mean that the clinical symptoms have abated. The effects of treatment can only be gauged by a careful examination of the stool for the presence of *E. histolytica;* for in amœbiasis, a temporary or clinical cure and permanent freedom from infection are unfortunately by no means always synonymous. Some authorities take cure to mean freedom from all symptoms and absence of amœbæ in the stools for at least six months from the cessation of symptoms. This is a difficult test to be of any practical value. The test for cure often laid down is six negative examinations during the three weeks following the treatment, the last three examinations being done in the third week. In the Carmichael Hospital for Tropical Diseases six negative examinations on six days after the cessation of treatment are taken as the criterion of cure and for all practical purposes this

Criteria of cure.

Three groups.

suffices; moreover, when the patients are relieved of all their symptoms they cannot be persuaded to stay in the hospital much longer. The treated cases are divided into three main groups.

(1) *Failure of treatment.* If either vegetative or cystic forms of *E. histolytica* are present, the treatment has failed.

(2) *Probable cures.* If the examination of six stools after cessation of treatment gives negative results the case is probably cured. These cases however cannot be said to be absolutely cured, but the chances are on the side of a favourable prognosis.

(3) *Indeterminate cases.* If however six stools have not been obtained the case is 'indeterminate,' and the majority of cases unfortunately fall under this head in actual practice.

It may be emphasized here that it is very difficult to prove that *E. histolytica* has been eradicated after treatment even if it has entirely disappeared from the stools. It is well known that both vegetative and cystic forms disappear from the stools for weeks in uncured cases after cessation of treatment and then reappear. Periodic sigmoidoscopic examinations are very helpful.

Immunity.

In experimental animals no immunity phenomena have been observed in *E. histolytica* infections. In man a previous cured attack does not prevent liability to a fresh attack. Immunity to infection appears to be dependent upon the resisting power of the individual.

According to Craig certain individuals are so resistent to infection by *E. histolytica* that infection to the point of definite symptomatology is impossible and spontaneous disappearance of the parasite may eventually occur. Long continued infection may lead to relative immunity so far as symptomatology is concerned, as people living in regions, where incidence of infections is high, show little symptoms and infection is rarely encountered in them. In such regions it is the new-comers who are almost always infected and show severe symptoms. That human system reacts to *E. histolytica* is proved by the fact that precipitins and complement-fixing substance can be demonstrated in the blood. Their relation, however, to immunity is not known. It is possible that such organs as the liver may possess some immunity as the amœbae may be present in the liver without abscess formation.

When actual symptoms of dysentery are present, the amœbæ are numerous and in full activity, and are amenable to the action of emetine. The reason is that the gut in these conditions is hyperæmic and emetine circulating in the blood will have ready access to the amœbæ. The cases most resistant to treatment are the indeterminate group which get relapse after relapse and fail to react to any drug. In these patients there is superficial ulceration or even considerable thickening and fibrosis of the gut; the amœbæ are walled in and it becomes difficult for emetine to reach them. When there is a co-existing infection with bacillary dysentery, especially of the Flexner group, the contents of the gut are very acid and there emetine is not so effective.

Why it is that entamœbæ cause symptoms in one individual and not in another is difficult to explain. The influence of environment on the vegetative forms of *E. histolytica* is an important factor; the stasis of the gut contents and their consistency have a bearing on the encystation of the amœbæ. It is possible that these factors play a part in the carrier problem and prevent emetine from producing its usual effect.

Sigmoidoscopy.

Whenever possible, examination of the ulcer with the sigmoidoscope should be done for diagnosis as well as to see the curative effect of the drug. This instrument is of great practical use in the diagnosis of the latent stages of amœbic dysentery. Ulceration may be present without producing any pain. A healed scar after treatment is a sign that the amœbic activity has disappeared, but when traces of lesions are seen in the small depressions or pits which stud the mucosa, the disease has not been eradicated.

Diet in amoebiasis.

Diet is of great importance in the early stages of treatment of acute dysentery. Less food means more rest for the inflamed intestine, but this should not be done at the expense of the patient's strength. For the first few days it is preferable to give fluid diet such as chicken broth, rice water, egg albumin or barley water. Later, milk diluted with lime water and barley water or citrated milk may be given if curds are being formed. When this is well borne pure milk, Horlick's milk, or Benger's food may be substituted. Some patients, however, develop a distaste for milk and cannot digest it and such things as rice water (kanji), liver soup, jelly, fruit juices, etc. may be given. Very strict dietary is not necessary for sub-acute and chronic cases. It is not necessary to restrict the diet of carriers during treatment, but such articles of diet as are irritating or leave a large residue, *e. g.*, vegetables, hot curries etc., should be forbidden. It is advisable to stop all alcohol and also smoking during the treatment. During the convalescent stage the diet should be gradually increased, milk, puddings of sago, arrowroot, rice, cornflower, custard, etc., being given in place of milk; later lightly boiled eggs and toast butter or a little soft-boiled rice, fish, and white meat should be added gradually also grapes, baked or stewed apples, banana, orange, etc.

In addition to diet restrictions, the patient should avoid chill and exposures. The patients who have suffered from amœbic dysentery, even though the infection may be eradicated, are always liable to attacks of looseness of the bowels, the gastro-intestinal tract being very susceptible to slight irritations for a long time after.

Manson-Bahr has laid down the following dietary for four weeks after active treatment.

PERMITTED. Porridge, eggs, fish (haddcock, plaice, cod, sole, or whiting, either boiled or fried), chicken (boiled or roasted), rabbit, game (pheasants, partridges, pigeons, etc:), milk puddings (rice, sago, semolina, ground rice), toast, rusks, or biscuits, brown bread, vita bread, vegetables (spinach, vegetable marrow, cauliflower, brussels sprouts, young carrots, or young turnips), stewed fruit (pears, peaches, or prunes), baked apples, bananas, grapes, oranges, grape fruit, plain cakes, fruit jellies, custard pudding or plain puddings. Red meat—such as underdone beef or mutton—is advocated for lunch, and such dishes as tripe, brains and sweetbreads are also allowed. Beverages—light wine or claret.

NOT PERMITTED. Cheese, new bread, potatoes, fats, suet puddings, rich cakes, pastry of all kinds, coarse fruit and vegetables (old turnips or carrots, cabbage), pickles, sardines and preserved fish. Beverages—spirits, beer or stout.

Prognosis.

Prognosis on the whole is good if the patient receives proper treatment. Even if the patient does not, the prognosis so far as danger to life is concerned, is good, but so far as maintenance of general health is concerned, it is not favourable as there is always the danger of hepatic abscess and other complications setting in.

In the carrier cases the prognosis is very good and there is every chance of eradicating the infection if proper and persistent treatment is given. There is also chance of spontaneous recovery but without treatment the prognosis in this class is uncertain in as much as good health cannot be insured.

Prognosis in those suffering from repeated attacks of diarrhœa is uncertain, but here also infection can be eradicated with some of the modern effective drugs.

Mortality in amœbic dysentery is stated by Craig to be over 5 per cent in epidemic in Chicago, which appears to be a high figure in view of the modern effective treatment. Prior to introduction of emetine treatment, it is stated to have varied between 20 to 40 per cent. Prognosis is less favourable in patients who have had frequent attacks of acute dysentery as it leads to a condition of chronic invalidism. Fulminant and gangrenous cases are fortunately rare in these days and in these the prognosis is hopeless. Prognosis with regard to eradication of disease is doubtful. Mortality in liver abscess in Calcutta was 14.4 per cent when treated with emetine and aspiration. When complicated with empyema, it is said to be as high as 77.7 per cent. Prognosis increases in gravity with the number of abscesses and patients with multiple abscesses usually end fatally; even with the best of treatment it cannot be less than 25 to 30 per cent (Craig). It is therefore, very important to diagnose and treat the pre-suppurative stage with emetine; even when operative treatment is necessary administration of emetine reduces mortality. Prognosis in lung abscess is not good on account of pneumonia or empyema setting in. In brain abscess it is hopeless.

Prophylaxis.

Amœbiasis is an important public health problem and its successful prevention and eradication depends on general sanitation, pure water supply and prevention of contamination of food from flies, etc. Chlorination of water does not destroy *E. histolytica.* Care should be taken with regard to eating of raw vegetables, fruits and food exposed to contamination. The question of dealing with carriers is important and they should not be allowed to handle food. Proper disposal of night-soil and protection of water supplies against fæcal contamination is of prime importance. Most of the epidemics are water borne. Carriers should be detected and treated.

(4) Chemoprophylaxis

Quinoline compounds.

It was found that travellers and tourists during their stay in the regions where amœbic infection was endemic sometimes contracted such infections which proved very troublesome and were difficult to eradicate. Naturally the idea of using some of the amœbicidal drugs as a prophylactic against amœbic infection came up. These drugs, however, could not be used in the case of permanent residents but may be given to people who are going to stay for a short period in an endemic area.

Diodoquin and Chiniofon are the two drugs that have been used for that purpose. Arsenicals although strongly amœbicidal cannot be used without adequate medical supervision because of their toxicity. Out of the two quinolin derivatives diodoquin is however, preferable since the prolonged use of chiniofon sets up diarrhoea while diodoquin does not cause any disagreeable symptoms even after prolonged use.

The dosage of diodoquin recommended for prophylactic purposes is 7 tablets in a day, i. e., 2 tablets after breakfast, 2 after luncheon and 3 after dinner. According to Craig even two tablets after breakfast and two after lunch are effective. This should be continued for 20 days. If the stay is to be prolonged, the drug can further be administered after an interval of a week while if the period of stay is less, the drug should be taken for 20 days without reference to date of departure. Sometime 1 to 3 tablets of Chiniofon have been taken at

the onset of an attack of diarrhœa and continued as long as the symptoms persist with good result

3. Secondary Amoebiasis

Amoebic Hepatitis and liver abscess

Next to involvement of the intestines, the liver is most frequently affected in amœbiasis. Acute amœbic hepatitis may occur at any time during the course of the disease and its incidence may be about 5 per cent. It may start during the acute stages of the disease or may suddenly start during the course of a remission. The patient has acute præcordial pain, or a dull painful sensation over the hepatic region made worse by movement. The temperature may rise to 104°F with a rigor and the patient has profuse sweating, dicrotic pulse, flushed face and furred tongue; the liver is enlarged (3 to 4 inches below the costal margin) and is tender; a leucocyte count of 20,000 to 30,000 is usually found. Acute hepatitis may subside in 3 or 4 days with or without any treatment or the condition may yield rapidly to injections of emetine. Amœbic hepatitis is probably due to massive invasion of the liver with *E. histolytica* and may occur years after all the intestinal symptoms have disappeared in people living in the endemic areas.

(1) Hepatitis and hepatic Abscess

Acute hepatitis.

Acute hepatitis is not easy to distinguish from acute cholecystitis but in the latter leucocytosis is not so marked. Association with obstructive type of jaundice is also not met with. Sometime pain is referred to the umbilical region or the right iliac fossa resembling a perforated gastric ulcer or appendicitis. Chronic amœbic hepatitis may be confused with any other forms of enlargement of liver, such as that resulting from cirrhosis, malaria, etc. It is often a precursor of hepatic abscess, and usually subsides with emetine therapy.

Hepatic abscess.

Amœbic abscess of the liver has been shown to definitely occur in intestinal amœbiasis and absence of amœbic infection in the intestines does not rule out amœbic liver abscess. It is especially common in Europeans (2 to 5 per cent of infections) and it was much more frequently seen in pre-emetine days. Abuse of alcohol is said to be a predisposing factor.

Pathology.

Amœba have been found multiplying in the lumen of some of the deeper veins and the liver is probably affected via the portal vein. On account of the anastomosis of lymphatics between the pleura and the liver, the pleura may also be affected. Amœbæ, on reaching the liver, may not obtain a footing and may be killed; if they do get a footing, they break up the liver cells, feed on them and multiply forming necrotic focii. Several primary centres may coalesce and form an abscess with cytolised liver cells in all stages of degeneration forming a gummatous material (reddish brown liver abscess pus); this is usually bacteriologically sterile though it may become secondarily infected with such organisms as *B. coli* and the streptoccocus. Liver abscesses are usually solitary, but may be multiple (in 30 per cent of cases). The left lobe is less commonly affected, but the abscess may increase and involve the whole of the liver. At any time, a liver abscess may become encapsuled by arrest of its growth; the pus then becomes absorbed and becomes like thick cream cheese in consistency. Calcification may finally take place. The walls of such abscesses are fibrous and smooth, but these changes only take place when amœbæ are destroyed as a result of the treatment or some other cause. An abscess may be seen externally when it is large or multiple. Local peritonitis may occur by contact.

Symptomatology.

The patient first feels an uneasy sensation over the liver and later there are sharp stabbing pains radiating towards the right shoulder, especially at night; in abscesses of the left lobe, pain may be referred to the left shoulder joint. A very tender spot may often be found over the right rectus muscle. Soon the patient gets rigors and fever, the tongue becomes furred, appetite is lost,, and complexion becomes muddy. The temperature is highest at 10 PM and normal or even subnormal in the morning; there is profuse sweating, insomnia, restlessness and loss of weight. The liver dullness may extend an inch or more above the normal level in the nipple line; deep inspiration may produce pain; the spleen is not enlarged unless malaria co-exists. There may be a pleuritic rub at the base of the right lung; decrease of breath sounds and inspiratory crepitations may be present in cases where the lesion is extensive.

The abscess may produce emaciation and death unless relieved by operation or it may burst into the right lung, pleura or into a viscus and may thus discharge itself (natural cure).

Rarely symptoms may be entirely absent until the abscess bursts, or it may be only detected to post-mortem. At other times, initial fever may resemble typhoid, which may later assume a quotidian or intermittent character. Swelling in the epigastric or the hypogastric regions resembling tumours and even varicosity of veins in epigastric region may be seen. Night sweats occur in more than 80 per cent of cases and enlargement of liver in 75 per cent. Mortality used to be 50-80 per cent in old days but owing to improvement in diagonosis and treatment it has been reduced to less than 6 per cent.

Differential

Liver abscess is frequently missed on account of the variety of symptoms that accompany it. It may be mistaken for malaria (rigors occur), kala-azar (night sweats occur), undulant fever, etc. It must be differentiated from gumma, tubercular abscess, malignant disease, pyaemic gall bladder, pleurisy, subphrenic abscess, etc. Evidence of previous ulceration of the large gut shown by sigmoidoscopic examination, discovery of *E. histolytica* cysts in the stools, leucocytosis (on an average 15,000 to 30,000), anæmia, and radiological examination may be helpful. The chief points in radiological examination are fixation and limitation of movements of the diaphragm on the affected side and sometime irregularity of outline, and increase in size or doming of the diaphragm on the right side. The cardiophrenic angle becomes less acute.

Aspiration with an exploring needle and drawing the pus makes diagnosis a certainty. As the inferior vena cava lies in that region, the aspiration needle should not be inserted to a greater distance than 3¾ inches from the chest wall. The needle should be inserted after local anæsthesia at the most tender and most prominent position of the liver. If there is a definite buldging in an intercostal space in the mid-axilliary line, that site may be selected, or the posterior part of the thorax at the angle of scapula may be preferable. The needle should always be inserted in an upward and inward direction, *i. e.*, towards the nipple, in which case it will tap the abscess in the upper part of the right lobe. While the needle is being pushed in, traction should be made on the plunger to draw in any pus which may be encountered. The needle should not be passed in a horizontal direction. The liver pus is chocolate coloured and is usually sterile; on microscopic examination it shows disintegrating leucocytes, degenerating liver cells, and cholesterin crystals. Liver function tests are not helpful.

Treatment.

Acute amœbic hepatitis yields very quickly to emetine injections, and improvement is noticed after injection of one grain for 2 or 3 consecutive days. After this, the same dosage is administered as in routine anti-amœbic treatment. Chronic amœbic hepatitis is also amenable to anti-amœbic treatment with injections of emetine or use of ipecacuanha powder. The patient should be put on a light diet, and aperients, especially sulphates of sodium or magnesium, should be given with caution. Heat should be applied to the part in form of hot fomentations and antiphlogestine or dry cupping and leeches may be applied over the affected area if there is much pain. Ammonium chloride in doses of 20 gr three times a day reduces the size of the liver. According to Manson-Bahr, pus may be absorbed after administration of EBI, but this is doubted by other authorities. There is no doubt that treatment with EBI and chiniofon is distinctly helpful in preventing abscess formation.

Aspiration.

This should always be preceeded by a preliminary exploration described above. A 2 per cent novocain solution is then injected and a medium or a full size needle is inserted. Yellow serous fluid, especially with fibrin suggests pleural exudate, but it may indicate an underlying hepatic abscess. Aspiration of fluid from the liver may be beneficial even if no pus is found (hepatic phlebotomy). Potains aspirator is preferred because it is safe.

Surgical measures.

Surgical interference is rarely necessary in these days. Transperitoneal and transpleural routes are used according to the location of the abscess and when the abscess is draining well the temperature comes down. If there is another abscess it should be explored and drained. Emetine should be given in form

of injections in doses of one grain each, both before and after the operation till a total of 10 to 12 grains have been given.

It is now recognized that emetine therapy can by itself promote absorption of pus from a liver abscess without surgical interference. Amœbæ, it is held, are killed in the liver by emetine. The abscess in the active condition has a zone of hyperaemia round it and so long as amœbæ are alive the flow is from this zone towards the abscess. As soon as the amœbae are destroyed by the action of emetine, the current as regards the abscess is reversed, and the result is absorption and extinction of the abscess. The absorption of sterile pus is in no way detrimental to the patient. There is no doubt that the incidence of abscess of the liver has considerably decreased since the use of emetine in amœbic hepatitis. Neither the gravity of the condition of the patient nor the size of the abscess are contraindications to treatment with emetine. The disadvantage is that the absorption sometimes takes several weeks and this may weaken the patient. The only indication for surgical interference is the presence of bacteria in the aspirated fluid. It is rare to find bacteria in unopened abscesses and when they are present the conditions may not be amœbic in origin but due to extension of suppurative process from the peritoneum or pleura.

Emetine as an hepatic stimulant.

That emetine has some stimulant action on the liver cells can be judged by the fact that it is excreted in the bile and that it has a beneficial action in other conditions where functions of this organ have been deranged. The writer has found emetine useful in chronic hepatitis of malarial origin, catarrhal jaundice and chronic congestion of the liver due to causes other than heart and kidney diseases. The cholagogue action attributed to emetine has not been confirmed by experiments. Four injections of emetine, 1 gr daily are usually effective in improving the condition whether due to amoeba or otherwise. Such doses do not produce any cumulative toxic effects.

Emetine, however, has been wrongly used in conditions simulating amœbic abscess of liver for want of proper diagnosis. The writer has seen cases of chronic malaria with hepatitis given many injections of emetine because the symptoms resembled those of amœbic hepatitis. Although rigorous scientific proofs regarding the existence of primary amœbic infection of the lung, brain and bladder are not forthcoming, emetine injections cure these conditions when *E. histolytica* is found in them. Amœbic cholecystitis has been proved by finding amœbae in the pus from the gall bladder.

Emetine treatment.

Emetine has a remarkable effect in acute amœbic hepatitis. When the liver tissue is liquified by the action of amœbæ and when there is no bacterial infection; the amœbæ are still active and are vulnerable to the action of emetine. Small intra-hepatic abscesses yield rapidly to emetine therapy and the necrotic tissue is absorbed.

The abscess cavity may be large and may contain ½ to 1 pint of pus, its walls are lined with necrotic tissue containing active amœbœ. Under such conditions aspiration combined with emetine therapy is useful, as the quantity of pus is probably too large to be absorbed. Here also the amœbæ are still vulnerable to emetine.

In the final stages the abscess cavity is very large and the amœbæ are dead because conditions are not favourable for their multiplication. Often in long standing cases, secondary infection has occured and in such cases emetine is useless; the abscess has to be opened and free drainage is essential.

Prognosis. With proper treatment with emetine the prognosis is good. In abscesses which have opened into the lungs, secondary infection is bound to take place. In these cases sulpha drugs or penicillin should be used.

Patients who have had hepatic abscess and have been cured have lived in tropical climates for years after without any untoward effects.

Summary. Pain in the hepatic region, fever and leucocytosis in patients suffering from amœbic dysentery are signs of amœbic hepatitis and beginning of abscess formation. As soon as these are discovered injections of emetine should be started (one grain daily) for not exceeding ten days. Usually after three or four days the symptoms completely subside if it has not gone beyond the stage of hepatitis. When abscess formation has commenced or even if an abscess pus formed, ten injections may produce resolution and absorption of the abscess. If symptoms continue surgical measures have to be adopted. Emetine is a specific drug in the treatment of hepatitis and there is definite evidence that the drug may cure large abscesses. It is only in cases where secondary infection has taken place that surgical interference is required. Here emetine therapy should be combined with aspiration and use of sulpha drugs or penicilin. Radical operation is only necessary in a small number of cases.

(2) Rare Amœbic Lesions

Lung Abscess. Lung is the next commonest organ involved by the amœbae. It may be attacked as a result of direct extension of the abscess from the liver as the infection may be conveyed by the hepatic veins. Rupture of a liver abscess is a more common source of infection which is the commonest accident occuring in 10 to 20 per cent cases of liver abscess.

The formation of the abscess is preceeded by development of pleurisy, shown by pain, irritable cough and quick shallow respiration. As the abscess advances signs of consolidation become apparent.

The abscess may rupture into the pleural cavity or into a bronchus. In the latter cases, the sputum is typical anchovy-sauce like and may contain amœbæ. The patient may succumb sooner or later or in favourable cases natural cure may result after discharge of the pus for a long period. Treatment on the same lines as for abscess of the liver.

Brain Abscess. Brain Abscess is a fatal complication of amœbic infection and is seen most frequently among Europeans. Male adults between 20 to 40 years are more commonly affected. Generally the victim has had lung or liver abcess which may have become cured even months before. The abscess is usually single, developing in one of the cerebral hemispheres.

Pathology is the same as in the liver abscess, the contents being generally bacteriologically sterile, and necrosed brain tissue attached to the thrombosed vessels containing large numbers of amœbae. Meningitis is generally absent.

The diagnosis of the nature of the abscess is often difficult. Symptoms are those of a bacterial abscess minus the toxic evidences of suppuration. The cerebral spinal fluid is not under tension because meningitis is often absent. The disease advances rapidly and generally terminates in death within 6 to 8 days. Rupture of the abscess into ventricules causes acute symptoms. Lumber puncture, blood examination, ophthalmoscopic examination and examination of stools plus careful elicitation of history of dysentery and liver abscess may help in arriving at the correct diagnosis. Treatment consists of administration of emetine, localisation and drainage of abscess.

Peritonitis. Chronic adhesive peritonitis localized in patches is seen as a rule in old standing cases Acute peritonitis which is a fatal complication, may sometimes be seen as a result of perforation of a deep ulcer, or rupture of a liver abscess. Treatment consists of rest, application of heat and surgical measures.

Appendicitis. Appendicitis may often occur as a result of extension of ulceration from the caecum. Definite diagnosis is, however, often difficult.

Other complications still rarely met with are splenic abscess, ovarian abscess, cystitis, parotitis, fistula and intestinal heamorrhage. The last named may be severe and recurrent due to liver abscess or independent of that from the intestines originally.

Appendicitis occurring in the course of amœbic infection is often controlled by injections of emetine and chiniofon. Invasion of appendix means that the caecum is involved and operative procedure in these cases leads to complication. Appendicostomy and cæcostomy once recommended, are valueless.

CHEMO-THERAPY OF AMŒBIASIS

4. Ipecacuanha Derivatives

Historical.

Ipecacuanha or 'Brazil root' was used in Brazil as a remedy for dysentery many centuries ago. In 1684, it was brought to Europe and an account of its antidysenteric properties was published. The remedy soon came into use generally.

The powdered bark was given in 2 drachm doses with tincture of opium to prevent nausea and vomiting, and beneficial results were obtained in dysentery, tropical hepatitis and other conditions. The value of the remedy as a cure for dysentery became gradually established and in 1912 emetine, the alkaloidal active principle, came into use instead of the root.

The root is obtained from two species of Cephælis, belonging to the natural order Rubiaceæ. (1) *C. ipecacuanha* or the Rio ipecac (also known as *Psychotria ipecacuanha*). This is the only form recognised by the British Pharmacopœia. (2) *C. acuminata* or Carthagena ipecacuanha has a thicker root, its annulations are less marked and it is cheaper. The plant grows about 30 centimetres in height and is found in most parts of Brazil.

Plantations were successfully started in India in the Nilgiris and at Mungpoo near Darjeeling. The British Pharmacopoeia (1932) required that the root should not contain less than 2.0 per cent of the alkaloids. The United States Pharmacopoeia allows both *Cephaelis ipecacuanha* (Rio) and *C. acuminata* (Carthagena) if yeilding not less than 2 per cent of ether soluble alkaloids.

Characters of the root.

From the slender root and prostrated stem, roots are given off at intervals; some of these develop an abnormally thick bark and this thickened bark and thickened root constitute the drug of commerce. The root is gathered during the dry season, quickly dried in the sun, and sifted from the adherent sand and earth. The official root is slender and tortuous, varying in colour from dark brick red to dark brown. It contains 2 to 3 per cent of total alkaloids of which two-third is emetine; ipecacuanha wood yields about 1 per cent of total alkaloids.

Ipecacuanha stem has often been found mixed up with the root and much of the drug that is imported is a mixture of the two. It contains 0.97 to 1.8 per cent of the total alkaloids, the yield being lower than that of the root.

The rhizome of a small monocotyledon plant *Cryptocoryne spiralis,* (Tamil-*Athi Vadayam*) has been exported from Southern India (Madras); the root of *Psychotria emetica* or the greater striated ipecacuanha; the roots of *Richardsonia;* the root of *Ionidium ipecacuanha, Trinidad ipecacuanha* which is the rhizome and root of *Asclepias curassavica. Naregamia alata* (*tin pani* or *titvel*) grows in Western India, are some of the substitutes and adulterants.

Test for emetine.

The following test for emetine is useful for distinguishing roots which contain that alkaloid from numerous substitutes.

Half a gm of the powdered root is mixed with 20 ccm of strong hydrochloric acid and 5 ccm of water and filtered. To 2 ccm of filtrate 0.01 gm of potassium chlorate is added; if emetine is present the liquid assumes a yellow colour changing to red in the course of an hour.

Chemistry of ipecacuanha.

In 1817 Pelletier isolated the alkaloid emetine; in collaboration with Magendie its physiological action was determined and in 1829 it was first used in the treatment of dysentery. The discovery of emetine was quickly followed by the isolation of several other important alkaloids strychnine, quinine and veratrine. Pelletier's emetine contained the total alkaloids of ipecacuanha and this name continued till 1894 when it was shown that it consisted of two alkaloids namely *emetine* and *cephaeline.* Subsequently the presence of

a third alkaloid psychotrine was demonstrated which occurs only in small quantities. These three alkaloids are chemically closely related.

Composition and characters of the alkaloids.

Emetine is a white amorphous substance which darkens on exposure to light, gradually assuming a yellow colour. Its halogen salts, nitrate are crystalline, the sulphate and the acetate is amorphous. The hydrochloride which is largely used is a white crystalline powder and is easily soluble in water and alcohol. Emetine is an isoquinoline derivative because the latter substance is formed on oxidation with potassium permanganate. It would therefore appear to be related to papaverine and narcotine; in fact some of the opium alkaloids have been shown to have a well-marked toxic effect on protozoa. Emetine is methyl-cephæline; iso-emetine is probably a stereo-isomeride of emetine, but attempts to convert one to the other have so far been unsuccessful. Iso-emetine is considered by some to be a methyl ester of iso-cephæline. Iso-emetine is non-emetic and comparatively non-toxic; it has, little effect in *E. histolytica* infections.

Cephaeline is a crystalline alkaloid less soluble than emetine; it is soluble in alkaline solutions and darkens on exposure. By methylation, it is converted into emetine. Cephæline hydrochloride in doses of 1/12 to 1/6 grain is said to be a more powerful emetic than emetine.

Psychotrine is related to cephæline but has 2 atoms of hydrogen less. It is the least toxic of the three alkaloids and has a doubtful therapeutic effect in amœbic infection. On reduction, psychotrine yields a mixture of cephæline and iso-cephæline; these on methylation yield emetine and iso-emetine respectively.

New alkaloids.

Pyman (1917) isolated two new alkaloids—*O-methyl psychotrine* and *emetamine* from Ipecacuanha roots and the presence of traces of two other alkaloids are also discussed by him. The former two alkaloids are closely related to psychotrine and emetine respectively.

Besides the three main alkaloids there is also another constituent, ipecacuanhic acid, which at one time was considered to be responsible for the action of ipecacuanha in dysentery.

The relative amounts of emetine in ipecacuanha from various sources are as following:—

Brazilian root	Total alkaloids 2.7 per cent, emetine 1.35 per cent.
Brazilian stem	Total alkaloids 1.80 per cent, emetine 1.18 per cent.
Columbian root	Total alkaloids 2.20 per cent, emetine 0.89 per cent.
Indian root	Total alkaloids 1.98 per cent, emetine 1.39 per cent.

A crystalline glucoside ipecacuanhin has also been found in the root.

(1) Emetine

(a) Pharmacological Action

Amoebicidal action.

Vedder (1911) found that emetine in dilution of 1 in 100,000 killed living amœbæ in broth cultures and pointed out that 1 in 10,000 dilutions killed *E. histolytica* in pieces of mucus in stools, while 1 in 100,000 solution rendered them quite inactive in 3 minutes. Emetine was also said to be lethal to free-living amœbæ in dilutions up to 1 in 200,000 if the exposure was sufficiently long. *Entamoeba gingivalis* is paralysed in culture by a 0.25 per cent solution in four hours. Rogers tried hypodermic injections of emetine hydrochloride in the treatment of amœbic dysentery with excellent results. Dale and Dobell (1917) demonstrated that even 1 per cent solutions failed to have any effect on *E. histolytica* obtained from scrapings of the intestine of kittens infected with dysentery. The action is weak so that the encysted and the more resisting forms are not affected whereas the vegetative forms are killed. Dobell and co-workers tested the action of ipecacuanha alkaloids on cultures of *E. histolytica* and found that emetine and cephæline of all the substances tested were able in very weak concentrations (1 in a million) to kill the amœbæ, if allowed to act for a sufficient length of time. They tested the effects of various drugs on cultures in fluid medium and made it clear that emetine even in dilutions weaker than those met with in the body after administration of a therapeutic dose had a toxic effect on *E. histolytica*, provided it was allowed to act long enough and the reaction of the medium was not too acid. Cephæline was also found to be definitely toxic for *E. histolytica*. Chopra and his colleagues found that *E. histolytica* present in mucus flakes lost their vitality and were eventually killed by emetine in dilutions of 1 in 200,000, provided the medium was alkaline.

Local action.

The drug is a local irritant. Applied to the skin in the form of liniment it produces redness and itching; 1 in 500 solution causes marked irritation of the mucous surfaces. Some individuals are extremely sensitive to these effects and in them, urticaria and dermatitis may be produced by systemic administration of emetine. Application of a 1.0 per cent solution to the abraded skin may produce a weal. When it comes in contact with the cornea it sets up a painful keratitis. Subcutaneous or intramuscular injections cause œdema and hyperæmia of tissues and extensive capillary hæmorrhages in the muscle fibres round the site of injection. Unlike the cinchona alkaloids, no necrosis of tissues is observed and the action appears to be mainly on the walls of the capillaries and arterioles. A violent irritation of the bowels can be set up by irrigation with a 1 in 10,000 solution of emetine hydrochloride. Half a grain taken by the mouth produces nausea, quickly followed by vomiting in about an hour; an hour later loose stools are passed accompanied by griping. Much larger doses than can be borne by the mouth, are tolerated by injection without producing nausea, vomiting and diarrhœa; the effect would therefore seem to be purely local.

Gastro-intestinal tract.

Chopra and his collaborators (1928) have shown that emetine inhibits the action of ptyalin even in 1 in 200,000 dilutions. Peptic digestion is, however, stimulated by concentrations stronger than 1 in 2,000, higher dilutions having no effect. Proteolytic and lipolytic digestions are inhibited by concentrations below 1 in 10,000; higher dilutions accelerate these. On the digestion of starch the action of this alkaloid is somewhat inhibitory. Emetine increases the tone of the non-striped muscle of the gastro-intestinal tract, the movements of the gut are stimulated, the effect being more marked as one passes downward from the stomach to the colon. It was also shown that the action was on the musculature directly and not through the nervous mechanism. Emetic effects of emetine are due to reflexes and central stimulation.

After injection, emetine can be detected in the stomach and intestines. Large doses of emetine given by injection cause swelling and congestion of the mucous membrane of the whole of the gastro-intestinal tract, which is often covered with mucopurulent secretion or studded with ecchymoses. In the dog, ulceration of the gut has been produced by giving emetine subcutaneously.

Action on circulatory system.

On the heart of both cold-blooded and warm-blooded animals emetine has a markedly toxic effect. It depresses the excitability and conductivity of the heart muscle and produces slowing and dilatation of this organ resembling that produced by chloroform. Both emetine and cephœline produce a marked dilatation of the coronary vessels. The heart becomes irregular, auricular and ventricular dissociation may be produced, and death may occur from ventricular fibrillation, the heart stopping in diastole. In animals intravenous injections of large doses produce a marked fall of blood pressure; after small doses the pressure soon regains its normal level. The fall of pressure is due partly to the direct toxic action of the drug on the heart, but vasomotor paralysis is an important factor especially after large doses. In man there is an appreciable fall of blood pressure to the extent of about 20 mm of mercury after an intravenous injection or after repeated doses by other routes. Characteristic emetine pulse (weak, rapid and compressible) and cardiac irregularities of various types are seen after a series of injections of this alkaloid. The isolated heart is slowed and weakened by both emetine and cephæline and it finally stops in diastole.

Respiratory system.

There is some depression of the respiration after subcutaneous injections of the alkaloid, but after intravenous injections the respiratory centre is stimulated and the frequency and the depth of the respiratory movements are increased. Small doses increase the secretion of the respiratory passages and thus act as an expectorant. To this is added a slight relaxation of bronchial musculature which makes the removal of mucus easier. If toxic doses are given they have a decided tendency to cause pulmonary congestion or hæmorrhagic pneumonic consolidation.

Nervous system.

In the frog, emetine causes a slowly advancing central paralysis. In mammals, neuritis is produced and there is general depression of this system giving rise to lethargy. The nerve cells especially those of the anterior cornua are damaged first and then the fibres degenerate; there is evidence that the motor fibres are specially picked out. Emetine has a powerful mental depressant action in man. Painful neuritis somewhat similar to that produced by alcohol has been observed.

Emetine is said to produce stimulation of the uterine movements, but in dilutions such as those occuring in the body after its administration it has little or no effect.

Absorption and excretion.

Emetine and the allied alkaloids are rapidly absorbed from the mucous membrane and the subcutaneous tissues, and are eliminated by the gastro-intestinal tract and by the kidneys. Elimination of emetine by these routes is demonstrated by the effect the drug

produces in urinary and intestinal schistosomiasis. A part of the drug appears to be excreted by the bile which probably accounts for its curative effect in hepatic schistosomiasis and liver flukes.

Excretion by the kidneys is discontinuous and prolonged. The drug appears in the urine 20 to 40 minutes after hypodermic injection of a therapeutic dose, but during the course of treatment not more than one-sixth of the drug is excreted by this route. Elimination proceeds by fits and starts, active periods alternating with periods when little or none is found in the urine. The elimination in patients who have received 0.15 to 0.58 gm in 3 to 6 days may last for 5 to 9 weeks, showing that it forms deposits in the body. In some cases the drug appears to have been retained for periods ranging from three months to a year. Large doses irritate and produce inflammation and albuminuria together with chloride and nitrogen retention. The excretion of uric acid is increased by emetine as it is by other gastro-intestinal irritants.

Toxicity of emetine.

By subcutaneous injections the lethal dose for dogs and cats varied from 0.003 to 0.005 gm per kilo body weight. If 0.003 gm is taken as the maximum tolerated dose (corresponding to 0.210 gm for a man of 70 kilo or 145 pounds), it is apparent that the adult dose of ½ to 1 grain is well within the margin of safety, provided the number of injections is restricted to 12 to 15.

With large doses, the changes produced in the tissues are of an acute nature, and immediate death within 24 hours may be produced with one single large dose. Doses of 0.05 to 0.075 gm per kilo in dogs, and 0.02 to 0.15 gm per kilo in rabbits, guinea-pigs, rats, etc., are rapidly fatal. There is probably no threshold of safety when the drug is taken continuously.

The toxic range of emetine for all larger mammals is 15 to 20 mgm per kilo of body weight. In man it is inadvisable to give more than 1 mgm per kilogram of body weight or a total of 10 mgm per kilogram. Emetine attacks all tissues and therefore is a general protoplasmic poison; change in the kidney, liver, heart and skeletal muscles are identical, all showing hyperæmia, cloudy swelling and degeneration of the cells. The nerves do not show any detectable pathological change till the parent nerve cells are in a state of advanced degeneration. The only lesion seen in transverse section of the cord is degeneration of the anterior horn cells. Chopra, Gupta and Roy (1935) show that intravenous injections of emetine (1 mg per kilo) in Belgian hares decrease the iodine content of the thyroid and fall in adrenaline content of the adrenals. This latter may account for the fall in blood pressure.

The physiological actions of emetine and cephæline are very similar. It has been shown that cephæline is more irritant and about twice as toxic and emetic as emetine; their parasiticidal action on the entamœba is about equal. It is generally stated that emetine has a stronger expectorant action and is a stronger nauseant than cephæline; its depressant action on the heart and its irritant action on the kidneys are more marked. Psychotrine is much less toxic but is likewise less parasiticidal. Ipecacuanhic acid, ipecacuanhin and various cephæline esters are more or less inert.

(b) Emetine in therapy

Historical.

Ipecacuanha has long been known as *Radix antidysenterica*. One of the chief uses of ipecacuanha is in the treatment of amœbic dysentery, and though emetine has largely replaced the powdered root, the latter is sometimes employed as a supplement to emetine injections. In old days, when ipecacuanha root powder in doses of 30 to 60 grains was used, it was difficult to avoid nausea and vomiting. Opium and morphine were employed to prevent those unpleasant accompaniments; powders were also used with keratin or salol coating which prevented the drug being dissolved in the stomach and allowed it to pass on to the duodenum, setting free the alkaloids in the intestines. These methods have now been superseded by the introduction of alkaloid emetine.

Rogers (1913) started hypodermic injections of emetine as a routine treatment of dysentery. Trials were initiated on a large scale and it was found that, though almost a specific for acute amœbic dysentery, it had little effect on the bacillary form. The oral administration of the powdered root was combined

with emetine injections as it was thought that by this method the bowel could be thoroughly permeated with the alkaloid.

It may be said that both emetine and cephæline, when given in adequate doses, produce a prompt disappearance of all the clinical symptoms of an acute infection with *E. histolytica,* and often a permanent disappearance of this organism from the stools. When given by the mouth, however, they produce nausea and vomiting and are often not retained. Emetine has therefore to be administered in the form of injections and it preferably should be given intramuscularly on account of the tendency it has to produce local inflammations and hæmorrhages after hypodermic injections.

Effectiveness.

According to Knowles (1928) the general consensus of opinion appears to be:—(*a*) that emetine injections are by far the most satisfactory immediate line of treatment of amœbic dysentery, but (*b*) that emetine therapy is generally a failure in the treatment of the carrier condition. It has been shown that primary malaria, as therapeutically introduced in the treatment of general paralysis of the insane, is a disease which is readily amenable to quinine treatment; only a few days of quinine treatment are necessary to effect a cure without relapses. On the other hand, the experience of all workers in the tropics is that established and relapsing malaria is very difficult to eradicate. Possibly a similar state of affairs exists with regard to amœbic infection of the gut; the prospects of eradicating the infection by emetine therapy may be much better in patients seen when suffering from the first attack of amœbic dysentery than in cases where the infection has become chronic and is well established. In a series of 32 acute and chronic dysentery cases which had 6 to 9 grains of emetine by daily injections of 1 grain each, the ratio of probable cures to failures worked out as 1: 1.7. The cost of treatment roughly estimated was 2 to 3 rupees per head. These results are similar to those obtained by other workers. Emetine injections undoubtedly cure an infection with *E. histolytica* and the alkaloid is more effective in acute amœbic dysentery than in cases of chronic amœbic colitis. In the majority of chronic infections, however, the injections do no more than clear up the symptoms and these patients generally become carriers.

Rogers (1929) recommended daily injection of 1 grain for not more than seven or eight days. He followed the treatment by 20 to 30 grain of ipecacuanha with 10 grains of tannic acid (to lessen the danger of vomiting) and a drachm of mucilage in one ounce of water, to be taken last thing at night before going to bed, and repeated each night for a week. By this method the organisms in the tissues as well as those in the bowels are acted upon by the drug.

Emetine and bismuth.

Deek's treatment consists in giving emetine injections combined with large doses (100 gr) of bismuth subnitrate orally. Knowles (1928) analysed a series of 55 cases treated with daily injections of emetine combined with one drachm of bismuth carbonate by the mouth every day. The patients were suffering both from acute and chronic symptoms, the majority belonging to the latter class. The ratio of probable cures to failures in this series was 1: 1.8. These results are not encouraging but there appears to be little doubt that bismuth treatment, in combination with emetine injections, from the clinical point of view at any rate, gives good results. The results so far as eradication of the infection is concerned are disappointing.

Emetine injections have also been used in combination with such other drugs, as *Holarrhena antidysenterica* bark, yatren, stovarsol, etc.

Treatment of acute cases.

Acute and sub-acute amoebic dysentery. The following procedure for treatment of such cases has been recommended by Acton and Knowles:—The patient is kept strictly in bed and made to use the bed pan. A saline purgative is given in the morning to flush the colon; some prefer to give an initial dose of castor oil or castor oil combined with opium (castor oil ½ oz or 15 ccm, tincture of opium 15 minims). The diet should be light, chiefly milk or boiled fish. Emetine injections are either given alone or combined with bismuth by the mouth, 1 to 2 drachms of bismuth carbonate is given every four hours in half a glassful of water during the day, with the object of decreasing the acidity of colon and increasing the alkalinity of the portal blood. Two and a half hours after the first dose of bismuth an injection of 1 grain of emetine hydrochloride is given. The timing of the injecton is important as it has been shown that emetine acts best in an alkaline substrate and that the alkalinity in the portal vein rises about that time. This treatment is carried on for nine consecutive days. For the next

three days emetine and bismuth are suspended and only a saline purgative is given. The complete treatment with saline, bismuth and emetine is again repeated for 3 to 6 days. The stools should then be examined for at least 6 consecutive days, preferably 8 or more. This is not always possible for the patient thinks he is cured as soon as the symptoms abate and leaves the hospital.

Treatment of subacute cases.

In case of acute or subacute types of amœbic dysentery, the writer has obtained very satisfactory results by the following line of treatment. The patient is kept confined to bed with a flannel abdominal binder and put on light liquid diet, preferably barley water or lime whey. Emetine is given intramuscularly or subcutaneously in 1 grain doses daily for six consecutive days. After an interval of three days, a second series of three injections are given in daily doses of 1 grain. Bismuth carbonate in one to two drachm doses thrice daily is given throughout the course.

In acute cases with severe pain and tenesmus give a hypodermic injection of 1/6 grain of morphia, or a small enema of opium and starch (tinct. opii 40 minims or 2.5 ccm, mucilage of starch 1 oz or 30 ccm). Hot applications to the abdomen such as turpentine stupes, or poultices consisting of linseed meal with a little mustard are soothing. The patient should be kept warm.

Failure in chronic cases.

Emetine in relapsing and chronic dysentery. Chronic and relapsing amœbic dysentery is one of the common diseases of the tropics and one of the most difficult to treat. Theoretically emetine should be an ideal drug against amœbiasis, as after injection the alkaloid is excreted in the gut and should thus destroy most of the amœbæ. In actual practice, however, it has not proved satisfactory and a large number of cases relapse. It is said that emetine cures about one-third of the cases; one-third apparently improve and in the remaining one-third the drug has no effect at all.

The reason for this failure in some cases is the fact that, although emetine acts on the amœbæ lodged in the tissues of the intestinal wall, it is said to have no effect on the parasites in the lumen of the gut. It is known that non-pathogenic amœbæ such as *E. coli* remain on the surface of the mucous membrane and possibly *E. histolytica* may do the same though, as a rule, the parasites are found in the tissues. They gain access to the radicles of the portal vein and are carried to the liver where they set up hepatitis and abscesses. The deeply situated organisms are only reached by emetine through the blood stream. Unfortunately in long-standing cases fibrosis is set up and the capillaries are cut off, thus preventing the access to the parasites of the drug circulating in the blood. Entamœbæ lying in the necrotic or fibrotic tissue (which is avascular) are thus liable to escape the action of emetine. This is the reason why many chronic cases are not amenable to treatment. The second factor is the infection of the ulcers with *B. coli* and other intestinal bacteria which further promote these pathological changes and lead to the chronicity of the lesions, thus hindering the curative action of emetine. Cases of bacillary dysentery both acute and chronic superimposed on a carrier condition of amœbic dysentery commonly occur in India.

Causes of failure.

Briefly the difficulty of curing chronic amœbic dysentery is due to four factors:—

(*a*) Emetine is a toxic drug and it is not possible to give it in sufficient amounts and over sufficiently prolonged periods to destroy all the parasites. Chronic infections are resistant to treatment though relief of symptoms occurs in the majority of cases. (*b*) Emetine cannot be brought into contact with amœbæ deeply seated in the ulcers by colonic irrigation. (*c*) The presence of

secondary bacterial infection is an additional factor in preventing cure. When amœbic and bacillary dysentery especially of the Flexner type co-exist, the reaction of the gut becomes very acid and emetine cannot exert its full therapeutic activity. In bacillary dysentery (Shiga type) the reaction of the gut may be alkaline to litmus (pH 8.11), in the amœbic form the pH of the stools is 6.3. It is found that when the pH of the contents of the gut is 7.0 the amœbæ are dead or dying; cyst formation occurs when the pH of the contents is 7.24; Charcot-Leyden crystals are passed at 6.96. The acid reaction hinders emetine which acts better in an alkaline medium. Attempts at markedly raising the alkalinity of the contents of the large intestine by giving alkalies by the mouth have not met with much success. The drugs which decrease the acidity of the contents of the large intestine are compounds of bismuth, preferably bismuth carbonate, which act by fixing the acids present.

Treatment of relapses.

If energetic treatment is not started in the early stages before extensive destruction of tissues has taken place, relapses are almost certain to occur. There is little doubt that the secondary bacterial infections probably account for most of the cases of failure with emetine. Resistant chronic cases of amœbic dysentery are sometime benefited with vaccine, made from such organisms as streptococci, dysentery bacilli, etc., previous to emetine injections. Emetine is much more efficacious in the early than in the later stages of dysentery. The drug is not active against the encysted amœbæ, which though themselves quite harmless, indicate active vegetative forms in the tissues. In cases of relapse, a course of emetine bismuth iodide, 3 grains daily for 12 days may bring about a cure. Dobell and co-workers recommend a 'double course' of this drug—3 grains (0.2 gm) daily for 24 consecutive days, but in the writer's experience in India very few persons can tolerate such large doses.

In the Carmichæl Hospital for Tropical Diseases, Calcutta, a course of 1grain of emetine daily for nine days was successful in curing a number of chronic cases of amœbic dysentery. The better method is combined treatment with emetine hydrochloride by injection and emetine-bismuth-iodide by the mouth. Emetine hydrochloride is given in one grain doses with emetine-bismuth-iodide 2 grains daily for 6 to 9 days. Even this proved a failure in a large percentage of cases. It has been shown that emetine fails to prevent cyst formation and encysted parasites remain in the gut for long periods.

Uncared cases and Carriers.

In a large number of cases the infection is not eradicated and often the cure is only an apparent one, cysts and Charcot-Leyden crystals being present in the stools. When infection is not eradicated the patient may suffer now and then from attacks of amœbic diarrhœa, or he may have constipation alternating with periods when he passes blood and mucus with stools. A patient in the carrier condition may at any time develop acute amœbic dysentery. An infected person frequently suffers from periodic attacks of acute amœbic dysentery when the large tissue-invading forms of *E. histolytica* are present in the stool. Between the attacks when acute symptoms have abated, the carrier condition is maintained. In such cases the courses of both emetine and emetine-bismuth-iodide may have to be repeated after a suitable interval, allowing for all the emetine to be excreted which usually takes 1 to 2 months after a course. Even then eradication of disease is not ensured. The drug treatment of amœbic dysentery should be controlled wherever possible by sigmoidoscopic in addition to microscopic examinations. (See Part II)

Autopsies carried out on amœbic dysentery cases, show evidence of the healing

power of emetine on amœbic ulcers. In the majority of cases a 9 to 10 days' course of emetine is sufficient to give relief and produce healing of the ulcers.

There is no doubt that patients passing *E. histolytica* cysts, especially those who show thickening and tenderness of the cæcum as a result of repeated attacks, should be submitted to repeated treatment with drugs because they have active amœbic lesions as can be demonstrated by the sigmoidoscope. Due care should be taken regarding the disposal of their fæces to prevent the spread of infection.

Various derivatives of emetine, such as dimethyl emetine, n-methyl emetine and iso-emetine, also act on histolytica infections. There is clinical evidence to show that the amœbæ acquire a tolerence to emetine after they have been subjected to its action for a long time (Manson-Bahr). Increased resistence of amœba in culture to emetine has been demonstrated.

Summary.

Emetine rapidly produces amelioration of symptoms of amœbic dysentery, *e.g.*, diarrhœa, tenesmus, etc., but its curative action in subacute and chronic cases is doubtful. Careful investigations have shown that it cures only a small proportion of cases. The motile and cystic forms disappear from the stools during the course of emetine treatment, but they often reappear later. The drug should, therefore, be used in daily injections of one grain each till acute symptoms have disappeared. For this purpose usually 4 to 8 injections are required and more than ten injections should never be given. Injections may be preferably combined with alkalies given a couple of hours before the injection, or bismuth carbonate may be given 2 or 3 times a day in doses of 1 to 2 drams.

If after that amœba are found in the stool, treatment with chiniofon or carbarsone or kurchi alkaloids should be given.

The patient should be strictly confined to bed when emetine treatment is being given.

Emetine should never be used in the treatment of chronic relapsing form of amœbiasis except a few injections to control the troublesome symptoms in the acute stage. It is a toxic drug with a cumulative action and more then ten injections should never be given in a course. The course should not be repeated in less than three months.

(c) Dosage and Modes of Administration

The treatment of intestinal amœbiasis with emetine must be continuous if it is to be efficacious. It must be given for a sufficient period and the dosage should be adequate. Intermittent treatment with small doses or only for a few days may relieve acute symptoms but will not eradicate infection; this may lead to a chronic condition which is very difficult to cure. On the other hand it should be remembered that emetine is a highly toxic substance and if given in large quantities it may produce severe diarrhœa, heart trouble, great prostration and even collapse and death. The consensus of opinion at the present time is that under no circumstances should more than ten to twelve injections of one grain each daily be given in one course, as they are almost certain to produce toxic symptoms. In the majority of cases 9 injections suffice. The total amount of emetine in one course should never exceed 0.01 gm per kilo body weight. The course can, however, be safely repeated after an interval of 3 to 4 months. For 6-months-old infants a dose of 0.01-0.02 gm, for one-year-old 0.03-0.05gm, total quantity up to 0.12 gm; for yet older infants up to 0.15 gm by injection. It should be given with great caution.

Dosage for infants.

Prolonged administration of relatively small doses of emetine is more to be feared than a few heroic doses and the immediate toxic effects of the drug are less dangerous than those due to cumulative action. The use of larger doses on alternate days has therefore been suggested, as this would enable the system to recover from the toxic effects of one dose before the next is given and thus make it possible to avoid further doses as soon as the earliest signs of cumulative action are recognised. In sufficient dosage with emetine,—less than one grain a day in adults—is liable to produce emetine—resistant amœbæ.

Modes of administration.

By mouth. Oral administration of ipecacuanha powder was recommended in old days to follow the course of emetine injections; 20 to 30 grains of the powder were given with 10 grains of tannic acid in a drachm of mucilage and an ounce of water, three hours after a light meal and before retiring. This was repeated every night for one week. The usual practice was to begin with a larger dose such as 30 to 40 grains (2 to 2.7 gm), then to decrease it nightly by 5 grains until a 5-grain dose was reached, which was continued for a week or longer if necessary. As a rule nausea and vomiting were constant accompaniments. About the middle of the course diarrhœa with canary yellow coloured stools appeared and this was looked upon as a favourable sign. In acute cases blood and mucus rapidly disappear and after the course the stool becomes normal. As the root powder contains many superfluous ingredients and is as irritant as emetine, it has now-a-days been superseded by the pure alkaloid.

Oral administration.

Emetine hydrochloride given by mouth is absorbed from the gut. Various devices have been adopted to reduce or abolish its nauseating or emetic effects. Unfortunately these have never succeeded without at the same time diminishing or annulling its therapeutic action. Pills and tablets coated with keratin, stearin, salol or other insoluble substances usually minimise the nauseating effects of emetine, but these may not dissolve in the gut and therefore produce no therapeutic effect. The best method of giving emetine by the mouth is as emetine-bismuth iodide.

Emetine has been administered in various ways, the object being to hurry its passage through the stomach so that the nauseating and emetic action of the alkaloid is avoided. Fuller's earth adsorbate or alkresta tablets have been recommended by some. Enteric-sealed tablets containing 1/3 gr emetine hydrochloride have been prepared by Lilly & Co. under the name of Enseals. These are designed to resist digestion for 3 to 4 hours and have given good results. Keratin-coated tablets have to be used at night before going to bed, the same precautions being taken as in the case of ipecacuanha treatment. Sometimes a certain amount of tolerance develops—the patient retaining the drug after rejecting it for the first 2 or 3 days; 10 to 15 minims of tincture of opium administered previously may diminish or abolish vomiting. As a rule in acute cases where the patients have to be kept in bed, injections are the best; in chronic cases with slight local lesions the oral method may be employed. Administration of ipecacuanha direct into the duodenum by catheter has been tried.

Rectal administration.

Good results are said to have been obtained by enemata of 4 to 8 gm of ipecacuanha powder suspended in a pint and a half of water. Colonic irrigations of 0.1 gm of emetine in 1,000 ccm of water or saline have been employed but they are very irritating, and painful. This method therefore is not recommended.

Subcutaneous and intramuscular routes.

Emetine is given either subcutaneously or intramuscularly. The drug is apt to cause an eczemetous condition of the skin if subcutaneous injections are

repeated in a limited area. Any source of focal sepsis should be eliminated as otherwise a fixation abscess may form. Deep subcutaneous injection is well tolerated, is less painful and is the method of choice.

The drug also is less irritant and painful when given by the intramuscular route owing to smaller number of sensory nerve endings there. Acton and Chopra (1924) showed that emetine injections in rabbits produced œdema and congestion of the tissues at the site of injection, and extensive petechial hæmorrhages but no necrosis. Some individuals develop an idiosyncracy towards the drug, a considerable amount of pain and stiffness being caused and even abscess formation may occur.

The symptoms last for several days, and even when injections are given into the loose cellular tissue of the abdominal wall, considerable redness and small hæmorrhages are found at the site of injection in spite of all precautions to secure proper sterilization of the skin and needle while other patients under the same conditions develop no trouble whatsoever. Pain caused by emetine is diminished by dissolving it in a 1.0 per cent solution of carbolic acid.

Intravenous route.

The intravenous route was rarely employed formerly but it has been used recently. As early as 1913 intravenous injections of emetine were tried, but toxic symptoms were not uncommon; these consisted of dyspnœa, vomiting, diarrhœa, very slow pulse, unconsciousness and muscular paralysis. Emetine by the intravenous route has been advised in the treatment of severe cases of amœbiasis; beginning with 0.03 to 0.05 gm on the first day, and reaching a maximum of 0.1 gm on the fourth day, a total of from 0.5 to 0.6 gm of emetine being given in 10 days.

The drug when given by this route should be well diluted and administered very slowly. The dose should not exceed 1/4 to 1 grain (0.015 gm) dissolved in at least 10 to 20 ccm of sterile saline a total of 6 to 9 injections being given. It is advisable to have an ampoule of adrenalin ready so that it can be given at once if there are signs of vasomotor paralysis. Intravenous injections are likely to be followed by nausea and vomiting which may supervene ½ to 1 hour after administration. The results are said to be rapid and striking and superior to those from intramuscular or subcutaneous injections. In the writer's experience intravenous injections did not give any better results than subcutaneous and intramuscular injections. Intravenous injections undoubtedly throw more strain on the heart and are not recommended.

(2) Compounds of Ipecacuanha and Emetine

As emetine, when given by the mouth, produces vomiting, so certain insoluble preparations which pass through the stomach unchanged without liberation of the alkaloid have been introduced.

(a) Emetine adsorbate or Alkresta Ipecac. (Lilly & Co.)

Alkresta ipecac is the trade name given to an adsorption compound of the total alkaloids of ipecacuanha with hydrated aluminium silicate (Fuller's earth). It is made up in tablets, each of which contains the total alkaloids of 10 grains of ipecacuanha. The advantage claimed for this form of emetine is that it passes through the stomach unchanged and the alkaloid is liberated in the alkaline intestinal contents. Knowles (1928) in a series of 7 cases found alkresta on the whole unsatisfactory.

(b) Emetine Bismuth Iodide

Composition and dosage.

Emetine-bismuthous-iodide (EBI) Dose 3 grains (0.2 gm) containing approximately 1 grain (0.06 gm) of emetine.

Du Mez (1911) manufactured emetine-mercurous-iodide and emetine-bismuthous-iodide and suggested, as results of his experiments on dogs, that these two compounds might be given in large and frequent doses, which would enable emetine to come in contact with entamœbæ for prolonged periods. Emetine-bismuthous-iodide is a brick-red powder, insoluble in water. In acidulated water it does split to a certain extent, as is evident from the fact that in 0.2 per cent solution of hydrochloric acid an opalescence is noticed. The double iodide is perfectly stable in air. It contains not less than 25 per cent and not more than 28 per cent emetine, 58 per cent iodine and 18 per cent of bismuth. Iodine is believed to reinforce the action of emetine.

It is believed that after administration of EBI, emetine is liberated from the compound on the surface of the mucous membrane of the gut, the insoluble bismuth salt being converted into sulphide after going through the pylorus. This reaction is probably slow and spreads over the mucous membrane. The underlying principle is that the drug decomposes in the large intestines and liberates "nascent emetine" which is very active against the amœba.

Before administering EBI, the patient should be strictly confined to bed, as unless rest is enforced, vomiting is certain to occur. Besides this, diarrhœa which invariably follows its administration, starts, and there is also danger of heart trouble. A preliminary dose of castor oil, one ounce, and tincture of opium 15 minims, is advisable. It is better to start with one grain doses and then gradually to increase to three grains. A total of 30 to 36 grains in a course was considered essential to eradicate infection formerly, but according to Manson Bahr smaller doses, such as 19 to 20 grains are quite effective, and doses not exceeding two grains daily are quite satisfactory.

It is advisable to give the double iodide as a loose powder in a hard gelatine capsule known as 'slipules' or paper cachet. It should not be given in hard or compressed tablets or mixed with insoluble excipients such as vaseline, soap, stearin, etc., nor should it be coated with keratin, salol or other more or less insoluble substances.

If a full dose of three grains is to be given it is better to give it in one capsule. Experience has shown that unless properly protected it is liable to be dissolved in the stomach giving rise to the usual irritant symptoms of emetine. Even keratin coating was found to be uncertain as nausea and vomiting were produced. In the presence of the alkaline contents of the duodenum the compound readily decomposes, loses its red colour and becomes opalescent white. A 3 grain (0.2 gm) dose of the insoluble salt containing about one grain of emetine, is the average dose and 10 to 12 such doses are to be given in succession. This is the standard 'full course' which was found to be successful. Indian patients can rarely stand more than 2 grains daily. Some prefer to give the drug in cachets of one grain each three times a day until 36 grains (2.4 gm) have been given; larger doses very often are not tolerated.

Vomiting when it occurs does not come on as a rule until some time after the dose, indicating the probable liberation of emetine from the intestine and not from the stomach. It is recommended by some authorities that the dose be given after a full meal when the stomach contents would certainly be acid, and on the whole, administered in this way, the drug is well tolerated.

Others prefer to give it on an empty stomach, and are of opinion that given in this way it causes less nausea. The dose should be given at night when the

Control of nausea.

patient is in bed and resting quietly, preferably with a cup of hot tea or arrowroot. The patient should endeavour to sleep and the saliva should be wiped from the mouth or expectorated and not swallowed. In many cases there is vomiting on the first night, but this does not matter if the powder is not rejected; on the second night there is usually little or no vomiting. Throughout the course there is a tendency to slight vomiting in many patients though a certain amount of tolerance does undoubtedly develop for the drug. If nausea or vomiting becomes troublesome it is necessary to give 15 minims (1.0 ccm) of tincture of opium, or chlorodyne, or omnopon 1/6 of a grain, half an hour before each dose. Hot applications in the form of a terpentine stupe or a mustard plaster to the epigastrium may also help. To obtain satisfactory results, reduction of the daily dose and intermission or shortening of the course of treatment must be avoided and treatment should only be stopped if severe prostration or cardiac depression supervenes. Bradycardia with nausea and low blood pressure, is an indication of intolerance to the drug. Emulsions of emetine-bismuth-iodide in 1 ounce of liquid paraffin have been tried and are said to have given good results.

Treatment of carriers.

As the treatment of the carriers with emetine or ipecacuanha during the first Great War gave very unsatisfactory results, emetine-bismuthous-iodide was investigated. Cats weighing 3 kilo could stand doses up to 40 mgm without vomiting. The drug was then tried in 10 human carriers, but it produced vomiting and diarrhœa. Dobell (1918) reported treatment of 11 carriers who had not been cured by injections of emetine. In all these cases the treatment was successful. He showed that an advantage is gained by administering emetine in the form of emetine-bismuth-iodide in chronic cases which have proved refractory to emetine. The effects of treament are in some cases remarkable and all stages of *E. histolytica,* whether cystic or otherwise, disappear from the stools. Emetine-bismuth-iodide was thought to be almost a specific against *E. histolytica,* and 2 or 3 courses were necessary to effect a complete cure. It should be noted also that if the first course fails to effect a cure a second course consisting of the same amount of drug administered for the same length of time is practically never efficacious. Both the dosage and the duration of treatment must be increased for the second course. Although undoubtedly failures after such courses have been reported, the chances of curing carriers of *E. histolytica* with this drug are greater than with emetine in the form of any other salt.

On account of its being a very irritant drug, a course of treatment with EBI, is very trying for the patient. Toxic symptoms are often produced inspite of all precautions. When this drug was first introduced the patients were kept confined to bed and a strict milk diet was enforced; this caused a general weakening and loss in weight. Experience has, however, shown that it is not necessary to diet the patient strictly and he can, with advantage, be allowed fish, chicken, milk puddings, etc. It is essential, however, that no solid food should be administered for three hours before the drug is given.

Uses in acute amoebiasis.

Emetine-bismuth-iodide is thought by some to be less efficacious in acute dysentery than injections of emetine hydrochloride, but Dobell and Low (1922) consider the compound to act equally well in acute cases, in carriers, and in all intermediate conditions. Lambert (1918) treated 40 Indian cases in Mesopotamia and came to the conclusion that in acute cases combined treatment, *i.e.,* emetine injection and emetine-bismuth-iodide by the mouth is the best. In relapsing cases and carriers emetine-bismuth-iodide alone is better. He considers that in emetine-bismuth-iodide we have a combination of considerable potency in the treatment of amœbic dysentery, preferably when the amœbæ are assuming their resistant stage. He recommends it in 2 grain doses every night for debilitated patients. Delayed vomiting indicates that the drug is beginning to take effect. Manson-Bahr and Sayer (1927) state that many cases which fail to respond to emetine injections respond to emetine-bismuth-iodide. They are of opinion that the introduction of this drug has considerably raised the percentage of cures in chronic amœbic dysentery. They admit, however, that such treatment is not ideal from the patient's point of view and there is need for a less toxic and more efficient

chronic amoebiasis. Effect in

drug. The well known toxic effects which appear after administration of emetine-bismuth-iodide are also an objection to its use. The degree of tolerance of patients to the drug varies considerably. In the writer's experience in this country, this drug has not proved satisfactory.

EBI acts in the same way as emetine does, but the consensus of opinion is that it is a more powerful amœbicide than emetine and its curative value is greater owing to possibility to re-inforcement by iodine present in this compound.

Diarrhœa.

Treatment must be regulated by carefully watching the patient who should be kept strictly in bed. In acute cases this is not difficult but patients who are carriers and have no definite symptoms may object. If rest is rigidly enforced there is less sickness and there is less liability to heart trouble. Emetine-bismuth-iodide generally produces diarrhœa about midway or a little later in the course; this is often beneficial as it keeps the bowel thoroughly cleared in addition to the specific effects of the drug. Towards the end of the course depression and general weakness are noticeable, and in some cases the heart sounds may become very feeble and irregular. The pulse should be felt daily and when this condition is detected the drug should be stopped. When the course is over the patient quickly recovers, diarrhœa ceases and the stools become solid.

Conclusion.

It will be seen, therefore, that while emetine-bismuth-iodide is a useful drug in chronic cases, it leaves a large residue of cases in which the infection is not eradicated even after several courses. The treatment with emetine-bismuth-iodide is drastic but is said to cure 70 to 80 per cent of cases of chronic carriers. When once a patient has resisted three courses of emetine-bismuth-iodide, it is no use persisting with it. Sometimes combination of the drug with yatren bowel wash (retention enema) or a vaccine made from bacteria infecting the ulcers may be useful. It may also be said that, the hope that the relative insolubility of the drug would prevent vomiting, which is a very annoying, tiresome and unpleasant complication in treatment, has not altogether been fulfilled. Emetine-bismuth-iodide is not so effective in the treatment of amœbic hepatitis nor can it be relied upon to prevent subsequent occurence of an amœbic abscess.

(*b*) *Emetine periodide* (E.P.I.). The dose is 2 grains (0.13 gm) thrice daily after food. The course lasts for 15 days. It is usually combined with 5 grains (0.3 gm) of exiccated ox bile which is said to increase its action. It contains 38.7 per cent of emetine and is insoluble in water. It is not, however, well tolerated as was thought nor is it as effective in eradicating infection as EBI. Emetine periodide aur-emetine, emetol and a number of other preparations of emetine have been prepared and tried with unsatisfactory results.

Summary.

Emetine-bismuth-iodide like emetine should never be administered unless the patient is strictly confined to bed. The toxicity of this preparation is like that of emetine and its dose and duration of treatment should be carefully regulated. It is often not possible to give the full dose of three grains daily to Indian patients and even doses of two grains daily are borne with difficulty. The usual course prescribed is three grains daily for 12 consecutive days and any reduction in dosage and duration is followed by unsatisfactory results. Often two or more courses have to be given and considerable strain is put on the heart; the course may have to be stopped because of cardiac weakness. When once started, the course has to be continued for 12 days to be effective. Nausea and vomiting are often troublesome. The drug is given by the mouth in hard gelatine capsules, or keratin or salol coated tablets at night after meals with a cup of hot tea or broth, the patient lying flat on the bed. Nausea and salivation occur

on the first and second nights generally and there may be vomiting, but after that toleration may be established. Diarrhœa occurs after 4 to 6 days and if excessive, the dose may be reduced but the whole course must be completed. After the course the patient is mentally depressed and weak. The patient should be confined to bed for 3 or 4 days after the course is completed and for 2 to 3 weeks he should avoid all physical exertion. The drug is not suitable for symptomless carriers or in abscess of the liver.

Total alkaloids.

Total alkaloids of ipecacuanha in amoebiasis. Roux (1923) employed subcutaneous injections of the total alkaloids of ipecacuanha with good results. In view of the fact that the amœbicidal action of emetine and cephæline is about equal, the use of the total alkaloids of ipecacuanha in the treatment of amœbic dysentery is worthy of trial. The cost of treatment could be considerably reduced if the total alkaloids could be employed instead of the purified emetine. Cephæline is more irritant and about twice as toxic and hence has never been widely employed.

Other uses of emetine.

Other uses of ipecacuanha and emetine. Ipecacuanha has been employed as an emetic and expectorant in the treatment of inflammatory conditions of the respiratory organs. For this purpose it is of course prescribed in very small quantities. Ipecacuanha increases the secretion of the bronchial mucous membrane. The increased secretion may be of service by protecting the inflamed and irritable surface of the mucous membrane from cold air and thereby lessening cough. The effects probably are purely reflex. It has also been employed as a diaphoretic. Emetine has been recommended for tuberculous hæmoptysis, but neither the clinical results nor its pharmacological action justify its use for any internal hæmorrhage; moreover some authorities believe it to be contra-indicated. Emetine has also been recommended in pyorrhœa alveolaris; it may produce improvement by destruction of *E. buccalis,* but it fails to cure this condition because this amœba only occurs as a secondary infection and is not the cause of the disease.

Emetine has been used in the treatment of a number of other diseases. It has proved useful in the treatment of metazoan diseases particulary in various types of schistosomiasis.

Acton advised injections of emetine in one grain doses in the treatment of leucoderma. He believed that in addition to its action on *E. histolytica* which is a common infection in these patients, the drug has a depressing action on the functions of the adrenals which are hyperactive in leucoderma.

Emetine in 1 grain doses has been given in sprue, but it produces no beneficial effects.

Emetine has been tried in *oriental sore.* Usually 20 minims of a 5 per cent solution of emetine hydrochloride in distilled water are injected at the base of the sore round its thickened margins. Inflammation is set up and in 3 to 4 days the sore becomes a well-defined ulcer which is then treated on surgical lines. Emetine has no specific effect either in dermal or visceral leishmaniasis; the effect is purely a local irritant one.

(3) Mode of Action and Toxic Effects of Emetine

In Vitro Action.

The manner in which emetine acts in amœbiasis has lately been the subject of much discussion. Vedder's observations that emetine hydrochloride in dilution of 1 in 100,000 inhibited the movements of *E. histolytica* were not confirmed by Dale and Dobell (1917). Quinine was found to be more toxic to the entamœba *in vitro,* yet it had not the slightest effect when administered in amœbic dysentery in large doses, whereas emetine was effective in very small doses.

Dobell and Laidlaw (1926) devised a technique by which the entamœbæ could be grown on a liquid medium and the action of different concentrations of the alkaloid was observed on the culture which was kept in an incubator for prolonged periods. With this technique, quinine concentrations lower than 1 in 1,000 gave cultures of *E. histolytica* as rich as the control. Stovarsol had no effect in dilutions of 1 in 20,000 or less. *E. histolytica* was very susceptible to emetine. In simple liquid medium 1 in 5,000,000 of emetine hydrochloride or 1 in 1,000,000 of cephaline hydrocloride was lethal for *E. histo-*

lytica in vitro within four days, provided the pH of the medium was about 7.0. With greater acidity of the medium the effect of emetine was much reduced. These experiments demonstrate that the dose of emetine or other alkaloids required to kill *E. histolytica* immediately bears no relation to the minimum dose of the same alkaloid which is eventually lethal, if maintained for some time. A very small quantity of emetine, if constantly present in the intestines for days or weeks, would probably suffice to make life therein impossible for *E. histolytica.*

It has been shown that changes in the cytoplasm of the amœbæ occur after an initial dose of one-sixth grain and that after four grains the nucleus of the organism becomes disintegrated. The point worthy of notice is that in order to eradicate the infection, one must aim at maintaining continuously over as long a period as is safe, a sufficiently high concentration to kill the amœbæ.

As regards the failure of emetine to kill entamœbæ in the cat, it has been suggested that man and the cat may deal with emetine in different ways. In the cat most of the emetine is probably excreted in the urine and not in the gastro-intestinal tract. Some human beings infected with *E. histolytica* appear not to react to emetine treatment. This may not be due to emetine-resistant strains, but possibly to the excretion of the alkaloid in larger quantities by the kidneys than by the gastro-intestinal tract.

Emetine action on tissues.

The other important factor lies in the tissues of the host, and we have to find out if any change takes place in these so as to make it impossible for an obligatory tissue parasite such as *E. histolytica* to penetrate the tissues and obtain the only food on which it can thrive and multiply. It is said that entamœbæ do not touch emetinised red blood corpuscles. Is it possible that the presence of emetine in the tissues of the host prevents this parasite from making use of them for its food? Or is it more likely that the presence of minute quantities of emetine prevents the entamœba by affecting its mobility or some other function from penetrating into the tissues or using them as food, and thus it dies of starvation?

Be that as it may, there is no doubt that when we attempt to exterminate the protozoal parasites from the body, the administration of a drug like emetine destroys a large majority of these parasites. The body resistance then rises and the patient's own power of resistance finally exterminate the residual parasites. This undoubtedly occurs in kala-azar and appears to hold good for malaria, trypanosomiasis and amœbiasis as well. Two factors, therefore, appear to be concerned in recovery, (1) the natural resistance of the host and his tissues; and (2) the amœbicidal action of the drug. A lowering of either of these allows the disease to progress.

Toxic Effects.

In adult human subjects the figures for immediate massive toxicity are not known. Fatal results however are to be feared with doses of 0.6 gm and anything above a dose of 1.2 gm is probably fatal at once. Eleven doses of 1.9 grains each would probably cause considerable damage in a man weighing 12 stone, while 35 doses of 0.8 grain each would be fatal. There should be long intervals (three months) between series of injections or courses, to allow the drug to be excreted from the body.

No general disturbances or gastro-intestinal symptoms are produced in man as a rule when a therapeutic dose is given by injection; local reaction as a rule is small when the solution is neutral. Larger doses cause nausea, vomiting and diarrhœa. As much as 0.25 gm has been given for a single dose without producing any other symptom except persistent nausea. But if a very large dose of emetine is given the patient may suddenly faint and death may occur from paralysis of the heart. In more slowly developing cases there is persistent nausea and vomiting, diarrhœa, vertigo, extreme muscular weakness and expiratory dyspnœa; the pulse is at first slow and then rapid. Death results from exhaustion, gastro-enteritis or intercurrent inflammation of the lungs. It should be remembered that there may be wide differences in the toxicity of various commercial samples of emetine.

Intolerance and Idiosyncracy.

Cases of intolerance and hypersensitivity to emetine do occur, the symptoms produced being marked weakness, hyperaesthesia, diminished reflexes, vomiting, slow pulse, abdominal pains and diarrhoea.

Cumulative toxic effects of emetine. Those who have had experience of treating amœbic dysentery are alive to the possibilities of the toxic action of this drug. Low, in his series of cases, noticed diarrhœa secondarily arising during treatment with emetine and suggested that its unduly prolonged use in too high dosages might eventually produce symptoms of intoxication. It is however very difficult to determine by clinical observation alone, whether the local effects on the alimentary canal and the general effects of toxæmia are to be attributed to emetine, or to the condition which had necessitated its administration.

Experimental Evidence.

Dale and Dobell (1917) performed some very interesting experiments on healthy animals to elucidate this point. They used chiefly rabbits and cats and employed doses which in proportion to the weight of the animals would be regarded as rather too high for continued administration in man. The average weight of an adult man being taken as 65 kilo a dose of 1.0 mgm per kilo body weight is equal to about one grain for such a patient. Cats weighing 3 kilo in these experiments were given doses of 5 mgm which works out to 1½ grains for an average man.

Such a dose given once only had no perceptible action of any kind on the animal and it could be repeated up to a point without any deleterious effects. If, however, the doses varying from 3-10 mgm were continued for a fortnight, sooner or later symptoms of intoxication appeared and became rapidly intensified with persistence of the daily injections, leading to a fatal termination. In rabbits, a profuse diarrhœa attended with rapid emaciation was the prominent feature; in cats these symptoms were of secondary importance and sometimes were altogether absent, but lethargy and somnolence were marked and deepened to a terminal coma. The toxic effects consisted of pronounced gastro-enteritis, acute nephritis, œdema of the lungs, weakness and paralysis of muscles. Post-mortem examination showed damage to the liver and kidneys in addition to signs of intestinal irritation; the heart was pale and flabby. The results are very significant and clearly show the cumulative toxic effect of the drug in these animals. They demonstrate the serious danger of pushing emetine beyond a certain point and ought to serve as a warning against the indiscriminate and unguarded use of the drug.

Clinical Evidence.

Clinical experience has also established the fact that emetine is a cumulative poison in man. The dosage and the length of time during which it should be given have therefore to be very carefully considered. Formerly, untoward effects from its use were put down to the disease itself and this accounts for such diagnosis as post-dysenteric heart failure. Recent investigations have shown that undoubted cases of sudden failure of the heart caused by emetine may occur. The depressing effect of the drug, if given in doses of one grain daily for some time, is very marked, the noticeable features occurring after 4th to 6th injections being loss of appetite, nausea, vomiting, abdominal pain and diarrhœa due to gastro-intestinal irritation.

Minor toxic effects are weakness of the calf muscles, associated with numbness of the soles of the feet, a kind of woolly sensation or a feeling of stiffness in the popliteal space.

Signs and Symptoms.

Among the serious symptoms of poisoning are an increased pulse rate, irregularity, listlessness and cardiac depression with fall of blood pressure. There may be general lassitude, disinclination to make an effort, weakness of the legs, tremors of muscles, globus hystericus, cardiac arrhythmia, low blood pressure and a feeling of faintness. General œdema, petechial hæmorrhages, purpuric skin rashes, desquamation of the skin, brittleness of nails, hæmoptysis and signs of cerebral and pulmonary œdema may also be present. Albuminuria sometimes occurs. Polyneuritis is common and in some cases difficulty in swallowing and

a feeling of constriction about the throat and chest have been noticed. Urticaria and large pruriginous plaques persisting for a month after the last injection have been known to occur even after a few injections. Sudden collapse and death may supervene in some cases. Auscultation of the heart in these cases shows similarity of sounds, and a lack of the muscular element in the first sound. Cardiac stimulants should be used at the earliest possible moment when the signs of cumulative toxic effects have appeared. Degenerative changes were noticed by Bais (1923) in human beings after prolonged use of the drug. Chopra and his collaborators (1924) demonstrated that in rabbits marked histological changes occur in the heart after emetine injections. These consist of cloudy swelling, disappearance of the transverse striations and atrophy and fibrosis of the muscle. The writer (1934) has shown that degenerative changes are produced in the myocardium after therapeutic doses of emetine which can be detected by electro-cardiographic examination.

Premonitory signs of poisoning.

The premonitary signs of poisoning for which a most careful daily examination is urged to avoid disaster, are:—(1) alimentary: persisting nausea, return of diarrhœa; (*ii*) urinary: oliguria, azotemia, slight albuminuria; (*iii*) Circulatory: grave hypotension, increased cardiac response to effort. The appearance of any of this triad calls for immediate suspension of emetine.

Emetine neuritis and myositis.

Neuritis of the lower extremities is one of the early symptoms and is manifested at first by weakness in the legs, difficulty in walking and interference with the normal reflexes. Usually there is no pain on pressure as the motor nerve fibres only are affected. Wrist drop, ankle drop, toe drop, and hyperæsthesia of the soles of the feet, sluggish knee jerks and loss of taste are noticed in some patients. Sometime one particular group of muscles may be affected and the condition may resemble early progressive muscular atrophy. The atrophy is preceded by myositis and muscular pains and probably a true myositis. It is possible that difficulty in swallowing and the sensations about the throat and chest produced after emetine injections are due to involvement of the nerves supplying those parts; it has also been suggested that these effects may be due to changes in the nuclei in the medulla oblongata similar to those that have been observed in the cells of the anterior horn of the spinal cord. In some individuals symptoms are produced after 8 or 9 grains of the alkaloid. Palsy may develop some considerable time after the cessation of treatment. Fibrotic changes in the nerve trunks have been noted after large doses of emetine in experimental animals, and similar changes probably occur in man. The effect of emetine, however, is more concentrated upon the muscle fibres rather than upon the nerve endings.

Emetine has not been found in the cerebro-spinal fluid, but it causes a slowly-advancing paralysis of the central nervous system in frogs. There is experimental as well as clinical evidence to show that peripheral neuritis is produced very early after a course of emetine injections. Emetine does not affect the nerve fibres indiscriminately but has a predilection for those conducting motor impulses. The weakness in the legs seen after emetine administration may also be due to damage of the muscle protoplasm.

Emetine diarrhoea.

Emetine when given in considerable quantities produces diarrhœa. It has already been pointed out that in animals emetine may produce gastro-enteritis with hæmorrhages in lymphatic glands, spleen, kidneys and thymus. These experimental animals die even though emetine is discontinued directly the symptoms appear. Diarrhœa which is the first sign of the gastrointestinal tract was quite common in the old days, the rationale of ipecacuanha treatment being to push

the remedy till profuse diarrhœa was produced and the stools became liquid and of a canary yellow colour. After discontinuing the drug the stool assumed its normal consistency, and to all intents and purposes the patient was considered cured. Whether the cure was real or not is uncertain as in those days cysts were not recognised. Low (1915) noticed that diarrhœa (occasionally with blood) occurred after hypodermic injections, but not so commonly as when ipecacuanha was given by the mouth. It is not unusual for the patient to get a slight attack of diarrhœa after the sixth injection. Since the oral administration of emetine-bismuth-iodide has come into vogue, diarrhœa has become more common, and when mild this is considered to have a beneficial effect and is not a sign of intoxication. In addition to the specific action of the drug, the bowel is at the same time thoroughly washed out and this mechanical process is valuable as is evident from the successful treatment of bacillary dysentery with saline purgatives. A few days after the termination of the course (3 grains of double iodide for 12 successive days) the stools commence to become solid and in successful cases gradually assume normal consistency.

Precautions.

Emetine is a protoplasmic poison acting equally on all tissues, heart failure being the actual cause of death. In cases where the heart or kidney is affected, it is advisable to give as small doses as possible for the treatment of dysentery. In otherwise healthy individuals the number of injections should be limited as far as possible, and under no circumstances should more than one grain be given in 24 hours.

Emetine, if administered continuously for therapeutic purposes, depresses the heart, but signs of cardiac depression improve when it is withdrawn. Caution is indicated in all cases where the myocardium is damaged by other diseases such as malaria (malignant form), influenza, diphtheria, etc. It is essential that the patient should remain in bed during the period of emetine treatment and an accurate record of the pulse rate should be kept. If this is definitely increased, or palpitation occurs, the drug should be withheld till the heart resumes its normal rate.

Convalescents who have received a course of emetine should be allowed to leave the bed very cautiously so that the effects of the drug pass off, and if the pulse rate goes up, they should return to bed. The writer has seen patients whose hearts were permanently strained for want of this precaution. Emetine is a powerful drug and appears to have a selective action on the heart muscle.

Emetine in pregnancy.

It has been suggested that emetine should be avoided in pregnancy as it may cause abortion. Some pharmacologists have stated that emetine increases the contractions of the uterus and have issued a warning against its use in pregnancy. On the other hand clinicians have given injections of emetine in advanced cases of pregnancy without untoward results. Recent investigations have shown that after a dose of one grain, assuming that the whole of the alkaloid is absorbed, the concentration of the drug in the blood cannot possibly be greater than 1 in 150,000 to 1 in 100,000. Such dilutions have little effect on the isolated uterus, and if there is any action at all, it is a tendency to relaxation. Acton and Chopra (1923) showed that abortion is more common in cases of bacillary dysentery and is produced by the bacterial toxins; they showed that the toxin of *B. dysenteriae Shiga* is a powerful ecbolic. Emetine does not appear to be a causative factor in producing abortion and the coincidence of a miscarriage when injections are being given seems to be due to the toxins of the parasite rather than to the drug. Pregnancy is not therefore a contraindication to the use of emetine. In menstruating women it is preferable to start the injections after the period is over but in urgent cases treatment should not be deferred.

5. Quinoline Derivatives

Since emetine is an isoquinoline derivative, other compounds of this group have also been tested for their amœbicidal action. More extensive investigations have been carried out with halogenated oxyquinolines and have resulted in the preparation of several compounds, yatren or chiniofon, diodoquin, vioform, etc., which have been used in the treatment of amœbic dysentery. The action of quinoline is reinforced by iodine. According to Craig (1944) "among the iodine compounds which have been recommended for treatment of amœbiasis, we possess the most efficient and safest therapeutic agents for elimination of this infection. Many iodine compounds have been recommended for this purpose, but the most efficient are chiniofon, diodoquin and vioform."

(1) Chiniofonum

Composition.

Yatren has been included in the B.P. under the name of CHINIOFONUM. It is also called quinoxyl. Chiniofon consists of a mixture of 7-iodo-8-hydroxy-quinoline-5-sulphonic acid, 4 parts, and 1 part of sodium bicarbonate; it contains 26 to 28 per cent of iodine. It is a light yellow odourless powder with a taste which is at first bitter and subsequently sweetish. Experiments on amœbic cultures show that the drug has a specific effect on *E. histolytica,* the effective range being between pH of 5.6 and 7.8 in concentration of 1 in 5,000. It also possesses high bactericidal activity without deleterious effect on the tissues, at the same time it acts as a cell stimulant.

The drug was introduced in 1921 and was marketted under the name of Yatren 105; it is identical with Anayodin which is the name given to it by another manufacturer.

Pharmacological action.

Chiniofon is claimed to be a cell stimulant and a strong disinfectant. The action of yatren on cultures of *E. histolytica, in vitro* has been studied. A solution of 1 in 100 kills amœbæ in a few hours; solutions of 1 in 1,000 inhibit multiplication in 12 hours and the cultures generally die out at the end of 24 hours with 1 in 1250. The amœbæ prove resistant to the action of 1 in 10,000 concentrations. Taken by the mouth chiniofon is a gastro-intestinal irritant and produces diarrhœa in large doses. Given intravenously in animals, a 5 per cent solution produced no fall of blood pressure or other untoward effects. Yatren is readily absorbed from the small intestine and from the rectum; it is excreted in the urine which gives a positive oxyquinoline test, *i.e.,* green colour with ferric chloride. Iodism is never produced though yatren contains 36 per cent of iodine.

Toxic effects.

Chiniofon is a non-toxic drug and as a rule produces no marked toxic effects in the doses in which it is administered in the treatment of amoebic dysentery. The only minor toxic effect produced is diarrhoea sometimes with a scalding sensation, which usually persists for two or three days and is not usually excessive. It is only in rare cases that it persists and the drug has to be stopped; the diarrhœa then disappears. In most cases decrease of dosage stops diarrhœa. In Indian patients more than 3.0 gm of yatren per day generally produce diarrhœa, but no other toxic effects were observed.

Toxic symptoms.

Two cases of death after intravenous injections of chiniofon were reported by Maxon (1933) and pathological changes were found in the liver, but the drug should not have been administered by this route.

Toxic symptoms are rare, but cases of acute or subacute fatty degeneration of heart, liver and kidneys have been recorded after intravenous injections. Compounds containing quinoline groups are said to be liable to produce necrosis of liver.

Craig who has very large experience with this drug has never observed any toxic symptoms except diarrhœa.

Chiniofon in Amoebic Dysentery.

Chiniofon has now been tried in amœbic dysentery in a very large number of cases extending over many years and according to Craig (1944) it is a safe and efficient specific in the treatment of this condition. Like all of the drugs

that have been advocated in the treatment of amœbasis, it fails in certain infections, but the records that are available and that cover over ten years of practical use of this remedy, show without question that it is curative in the majority of amœbic infections, when properly administered and that serious toxic symptoms never occur.

Although it can be given by the parenteral route, it is given generally by the mouth or in form of enema.

Course of treatment.

According to Craig (1944) Chiniofon is given in pills or tablets, each containing 0.25 gm (4 gr). In adults three of these are given three times a day after meals for 10 to 11 days (100 tablets). This dose generally produces some diarrhœa and if it is too severe, the dose may be reduced and then gradually increased to full dosage. The diarrhœa, unless severe, is beneficial in eliminating amœba from the intestines. The drug may be administered while the patient is doing his ordinary work and no particular precautions with regard to diet, etc., are necessary except that rich food should be avoided and alcoholic beverages are forbidden. In children the dose should be given according to age and general condition.

A course of 10 days treatment is usually successful in eliminating *E. histolytica* in carriers and mild symptomless infections, but if not, the course should be repeated after ten days. In patients with history of many diarrhœal attacks, it will be necessary to repeat the course of treatment once or twice before the infection is eliminated as some of these patients show entamœba in their stools after the first course or have a parasitic relapse later.

Combined treatment with emetine.

In acute and chronic amœbic dysentery, emetine is first given till symptoms subside and stools become semiformed or formed, not exceeding ten injections of one grain each; usually not more than 4 to 8 injections are required. After this a ten days course of chiniofon as stated above is given.

If chiniofon alone is used in acute amœbic dysentery, the use of high enema with the drug is combined with oral administration in doses of 0.5 gm (7.5 gr) three times a day. The daily enema is best given at night, consisting of 200 ccm of a 2 per cent solution in warm water; this should be retained for several hours, if possible. This treatment should be continued for 8 to 10 days, the patient remaining in bed.

After convalescence is established the stools are repeatedly examined for the presence of amœba and if positive the treatment with chiniofon is repeated.

Retention enema.

According to Manson-Bahr satisfactory and permanent results cannot be obtained when the drug is administered by the mouth alone. In doses of two four grains (0.25 gm) pills three times a day, it may produce considerable diarrhœa and fails to eradicate the infection. It is much more efficacious when it is given per rectum. Given in form of retention enemata, the drug appears to be absorbed as is shown by its excretion in the urine in 24 hours; the oxyquinoline test (green colour with perchloride of iron) is positive. He describes the following technique of administration of retention enema.

Technique.

Technique of Retention Enema:—The bowel should first be washed clear of mucus with a pint of 2 per cent sodium bicarbonate solution administered in the form of an enema. After an hour's interval, the patient lying on his left side with the buttocks raised on a pillow, 200 cc of 2.5 per cent solution of chiniofon in warm water is run in slowly through a funnel and stout rectal tube. The patient is encouraged to retain the

solution as long as possible, changing on the dorsal decubitus and right side after every five minutes intervals, thus allowing distribution of the solution on all sides of the large intestine. The solution after retention for several hours is then passed out.

The great danger associated with rectal medication that should be borne in mind is that the bowel wall may be friable. The mucosa is ulcerated, and perforation may occur especially when high enemas are given with a rectal tube or catheter. Even small enema (200 ccm) given under pressure may do this.

Value of Chiniofon.

Chopra, Sen and Gupta (1937) gave Yatren in the form of pillules, 4 pillules (1.0 gm) twice daily for ten or if necessary for fifteen days. The original dose proposed was 1 gm, thrice daily, but Indian patients could not tolerate this on account of the diarrhœa which was produced. Diarrhœa can, however, be controlled by strict diet and rest in bed. If the sigmoidoscope showed the presence of ulcers, a 2 per cent solution of yatren was employed for rectal lavage. The ulcers healed up rapidly after contact with yatren solution. Cure was recorded if six examinations of the stools on different days after cessation of treatment revealed no entamœba. No untoward symptoms were observed as a result of the treatment; diarrhœa might come on after the second day but was rarely troublesome, though it continued till treatment ceased. Out of 50 cases who were given this treatment 28 were cured and others left before the requisite number of stools could be examined. The percentage of cures both in cyst and trophozoite passers was 60.4; cure rate among cyst passers was proportionately higher. The drug is, therefore, certainly worth trying in amœbiasis of the intestine, whether acute or chronic, and is of special value in patients with renal or hepatic conditions, and in cases of dermatitis in which carbarsone is inadvisable. There is proof that yatren introduced per rectum percolates through the large intestines. In a patient who had retention enemata of 2.5 per cent yatren and who died suddenly of coronary thrombosis, autopsy showed yatren solution coating the mucosa of the large intestines. After a ten days course of yatren by the mouth and per rectum, relapses are frequent, but the effects are good in those cases which have become resistent to emetine and EBI.

In view of the above a combined treatment with EBI and chiniofon (yatren) per rectum has been recommended.

It should be remembered that this form of treatment is troublesome to the patient and can only be carried out properly in nursing homes and in large hospitals. Some clinicians have given the drug by intravenous injections but the results have not been superior to those by the other routes. Yatren has also been advised in cases of amœbic hepatitis or even liver abscess, but it has no marked effect. Patients suffering from bacillary dysentery derive benefit from it.

Combined treatment.

Combined EBI and Chiniofon treatment:—Chiniofon is undoubtedly of value in the treatment of chronic cases of amœbic dysentery or carriers, in combination with emetine injections or emetine-bismuth-iodide by the mouth. It has been abundantly demonstrated that both these drugs possess a definite curative value in amœbiasis, which may be limited when they are used singly. Chiniofon is readily absorbed but it is possible that it may not reach all the lesions in the bowels in sufficient concentration to kill the parasites. Its action may be intensified when it is combined with emetine by injections. The combination is well tolerated and gives very satisfactory results.

The patient is put on light diet and a ten days course is given. A retention enema is given at 8. 30 A.M. with the technique above described and retained till 5.0P.M. if possible. When it is voided EBI is given in doses of two grains at night before going to bed and a total of 15 to 20 grains need not be exceeded. If emesis occurs one grain of luminal may be taken half an hours before EBI is taken. Chloretone in 10 grain doses, tincture of opium 10 minims, or allonal, two tablets, the patient needs a few days complete rest. If relapse occurs, a further course should be given, but the strength of chiniofon should be increased in the enemata to 5 per cent and if necessary the combined course may be repeated again. Manson-Bahr considers this the most effective treatment of chronic

amœbiasis yet devised if it is conscientiously carried in all its detail. Even with this, a small residue of uncured cases is left.

(2) Diodoquin

Composition.

In diodoquin the sodium sulphonate radical of chiniofon has been replaced by a second iodine atom, thus forming a double iodine compound with the formula 5-7-diodo-8 hydroxy-quinoline. The compound is tasteless, very insoluble in water, dilute acids and alkalies, and only slightly in common organic solvents. It contains 63.9 per cent of iodine (Chiniofon contains 26 to 28 per cent, Vioform 41.5 per cent). As the efficacy of these compounds is partly due to iodine, it was thought it would be very effective. Its insolubility, however, acts against its effectiveness to some extent.

Craig investigated this compound and found that the dose recommended by the manufacturers was too small to produce any effect on amœbic infection in dogs; the dose was, therefore, increased and the drug was found to be an excellent and effective non-toxic amœbicide.

Dosage.

Diodoquin is supplied in tablets of 0.21 gm (3.2 gr) and its dose should vary with the severity of the infection. In carriers and patients with mild symptoms 6 to 7 tablets are given in divided doses after meals and continued for 20 days, while in more severe cases 8 to 10 tablets are given. The course may be repeated after 7 to 10 days rest with the same or increased dosage. No toxic effects or irritations of the bowel were produced. Large doses, such as three tablets three times a day for 20 days, giving a total of 1.84 gm (28.8 gr), were used with good results and no toxic symptoms. The drug probably has prophylactic value also and Craig recommends two tablets after breakfast and two after dinner and considers these will suffice to kill any amœba ingested.

The drug may be used in place of chiniofon in the treatment of symptomless carriers and mild infections, and may be preferred in children as it produces no diarrhœa. Two tablets three times a day for twenty days are recommended as the usual course.

In amœbic diarrhœa it is believed to be superior to chiniofon in doses of three tablets three times a day. Excellent results have been obtained in acute amœbic dysentery.

(3) Vioform and Enterovioform

Vioform is iodochloroxy quinoline and contains not less than 37.5 per cent and not more than 41.5 per cent of iodine and not less than 11.5 per cent and not more than 12.2 per cent of chlorine. It is a greyish-yellow powder with a faint aromatic odour, almost insoluble in water and sparingly in alcohol. It was used as a substitute for iodoform and was introduced in therapeutics in 1932.

Toxicity.

In therapeutic doses its toxicity is low and even with 100 mgm dose per kilo for 10 days, rabbits showed no pathological injury. In a single dose of 250 mgm, there was fatty degeneration in the liver with necrotic areas and some injury to the renal tubules.

No toxic effects were noted in man with therapeutic doses, except rarely, headache, palpitation, dyspnœa, colic, or diarrhœa with mucus and blood in the stools.

In the treatment of amœbiasis it is given in doses of 0.25 gm (4 gr) tablets three times a day for ten days. After an interval of one week another course of 10 days is given. It is not as effective as chiniofon. *Dosage.*

The drug is given by the oral route in gelatine capsules. On account of its irritant action it cannot be used per rectum. In chronic cases and in severe infections larger doses may be used.

The basis of enterovioform is vioform which is combined with saponin to emulsify it. This compound is said to be useful in the treatment of carriers passing cysts. Two tablets of the compound containing 0.25 gm are given three times a day after meals for a period of 10 days and the course may be repeated after one week. No toxic symptoms have been observed. *Enterovioform.*

The writer has used this drug with good results; it certainly ameliorates symptoms.

6. Arsenical Compounds

A number of aromatic compounds of arsenic have been used in the treatment of amœbic dysentery of which Carbarsone is the best known.

(1) Carbarsone

4-Carbaminophenyl arsonic acid or carbarsone is a white crystalline solid, tasteless and odourless, almost insoluble in water, but soluble in alkaline solutions. It contains 28.85 per cent of arsenic. After oral administration it is absorbed and is excreted in urine. It is the least toxic of the three arsenical compounds used in the treatment of amœbiasis. *Composition and dosage.*

Clinical experience has shown that in therapeutic doses it produces no serious toxic effect, but since it contains a substituted amino group in para position it must be used with great caution. Toxic doses in animals produce lethargy, diarrhœa and renal damage. In man in a large series of cases when the drug was given in doses of 0.25 gm (4 gr) twice daily for 20 days, it produced no toxic effects except slight gastric distress.

The usual course given by the writer is 0.25 gm (4 gr) twice daily for ten days. In resistant cases a 15 to 20 days course is given. The drug is given by the mouth in form of tablets or in gelatine capsules. In the writer's experience the use of enemata along with the oral route gives no advantage. The course of carbarsone should not be repeated before three months are over from the date of completion of the last course. *Course of treatment.*

There is, as a rule, no irritation of the stomach and the drug is retained without any difficulty.

It is claimed that carbarsone is effective if given per rectum as a 1.0 per cent solution in 200 cc of a 2 per cent sodium bicarbonate solution. When such retention enemas are given, a rapidly acting sedative, such as sodium amytal may be given in 3 grain doses (0.2 gm) to produce sleep so that the patient can retain the drug. If enema is rejected it should be repeated and at least five successful enemas are advised. This method of administration, in writer's opinion, does not eradicate infection.

The action of the drug is due to toxic effect of arsenic on the amœba. According to Craig, carbarsone can be relied on to cure 85 to 95 per cent of the mild

infections. He, however, believes that arsenical preparations should be used in those cases which do not respond to the quinoline and iodine compounds.

Effectiveness.

The writer has found that the drug is much more effective if a small dose of a mild saline purgative, such as Siedlitz powder or fruit salt is given every morning while the drug is being taken. Chopra and his co-workers (1933) tried carbarsone in a number of cases of chronic amœbic dysentery with vegetative and cystic amœba in the stool; 0.25 gm of the drug was given twice daily for 10 to 20 days, the usual course being for 15 days. A mild saline purgative was given daily whilst the drug was being administered; the criterion of the cure was six negative examinations during a fortnight following the treatment. In a series of fifty-three cases treated it was noted that the stools, as a rule became free from amœbæ three to four days after the beginning of treatment. The proportion of probable cures to failures was 1 : 0.12; no toxic reactions were noted, while the general toxic effect common to aresnicals was not very pronounced.

Toxic Reactions.

A complete absence of toxic reactions has also been noted in patients given from 150 to 1,200 mgm per kilogram of body weight over a period of forty-eight weeks. A number of mild reactions have, however, been reported in human beings, ranging from nausea, vomiting, diarrhœa, headache, sore throat, lachrymation and sneezing, to such severe symptoms as generalised pruritis and exfoliative dermatitis, laryngeal and pulmonary œdema, retinal œdema and papillitis, granular casts and albumin in the urine, and icterus. The drug contains a substituted amino group in para position and must, therefore, be used with caution. It should not be used in the presence of kidney or liver disease and is, therefore, contra-indicated in amœbic hepatitis. Certain cases of dysentery fail to react either to carbarsone or to any other amœbicidal drug.

Other arsenicals.

Amibiarson.—Chopra, Sen and Sen (1935) reported on the use of a compound termed 'Amibiarson' which is said to have a chemical constitution very similar to that of carbarsone, and like it, is given orally in doses of 0.25 gm in gelatine capsules, twice a day for from ten to fifteen consecutive days. The patients remain on an ordinary diet and a saline purge is taken with the drug.

Of forty patients who received this treatment, twenty-five were cured; in ten the results were indeterminate and in five the treatment failed. The ratio of probable cures to failures was, therefore, 5 :7.1.

Stovarsol, also known as *acetarsol,* is acetyl aminohydroxyphenyl arsonic acid. It contains from 27.1 to 27.4 per cent of arsenic. Craig used this drug largely before the more effective and safer compounds with quinoline and iodine were used. This drug is definitely more toxic than carbarsone or treparsol. The writer's opinion is in accord with this and does not advise its use in amœbic dysentery.

Treparsol.—This is an arsenobenzene derivative and is meta-amino-para-oxyphenyl-arsenic acid. It is marketted in form of 0.25 gm (4 gr) tablets and excellent results were reported from its use in amœbic dysentery in doses one tablet three times a day for four days, a rest of 10 days and another course of 4 days. The tablet should be chewed with food so that arsenic is widely dispersed in the intestine.

Indiscriminate use of the drug is not without risk but as this drug is rapidly excreted, it is preferred to stovarsol. The toxic symptoms are chiefly erythema, nausea and vomiting.

As in case of all arsenical drugs, the drug should not be used in patients with disease of liver and kidneys. Craig prefers treparsol to stovarsol, but not to quinoline-iodine compounds.

Treparsol, an arsenobenzene derivative has also been used in combination with emetine with good results.

7. Holarrhena Antidysenterica

It is a small deciduous plant belonging to the natural order Apocyanaceæ. It is also known by the name of *H. codaga, H. pubescens, H. malaccensis, Wrightia*

antidysenterica, and *Chenomorpha antidysenterica.* Two species *H. congolensis* and *H. Wulfsbergil* growing in West Africa also have a reputation in the treatment of dysentery. In England it is known as bitter oleander, dysentery rosebay, oval-leafed rosebay or conessi bark tree. In Sanskrit it is called *Indrayav* or Indra's seeds, *Kutaja* and *Kulinga;* in Hindi, it is known as *Indrayaba, Kureya* and *Dubhi;* in Bengali, *Kurchi* or *Kutrai;* in Persian, *Indrajavatalkh.* The bark is known as kurchi bark or conessi or Tellichery bark.

Distribution.

The plant is widely distributed throughout the plains of India and Burma in deciduous forests and open waste land, where it is often gregarious. It grows in the valleys of the outer Himalayas up to a height of 4,000 feet above the sea-level, and is abundant in the sub-Himalayan regions. It is also common throughout the greater part of the Indian peninsula down to Malabar and Travancore and grows abundantly in the forests of Burma. *H. antidysenterica* is often mistaken for another species of the same family, *viz., Wrightia tinctoria,* in fact Linnæus was originally responsible for this confusion. In 1809 Brown revised the whole of the family of Apocyanaceæ and rectified the mistake. In spite of this the two plants have been mistaken for each other. *Wrightia tinctoria* is inert and it is largely used owing to this fact that *H. antidysenterica* did not attract much attention, because more often than not the inert bark was used instead of the active Holarrhena.

Both the bark and seeds of this plant are among the most important medicines of the Hindu materia medica; infusions made from these have been used in the treatment of dysentery for many centuries. The Hindu physicians consider the plant to have bitter, stimulant, antipyretic, astringent and antidysenteric properties. It is said to stop hæmorrhage, and is an expectorant and is used as a tonic. It forms part of many preparations in the Ayurvedic (Hindu) and Tibbi (Mohammedan) medicine.

Seeds. The seeds contain 29.36 per cent of a fixed oil and 0.025 per cent of the alkaloids.

Chemistry.

Bark. As early as 1858 Haines isolated an alkaloid which he called *nereine* from the Linnæan name of the plant, *Nerium antidysentericum.* In 1864 Stenhouse isolated an alkaloid from the seeds which he called *wrightine* after the then commonly accepted name *Wrightia antidysenterica.* Both these names were rejected in 1865 by Haines who called the alkaloid *conessine.* Ramchandra Dutt (1880) gave the name of *kurchicine* to the alkaloid after its Bengali name. The same alkaloid occurs in the two African species mentioned above and Pyman (1919) isolated a new alkaloid to which he gave the name of *Holarrhenine.* Ghosh and his collaborators (1928) found in the Indian variety a second alkaliod in addition to conessine differing from holarrhenine which they named *kurchicine,* and a third alkaloid with a low melting point named *kurchine.* Assay of the powdered bark shows that it contains 1.5 per cent. of total alkaloids by weight; but it is difficult to get conessine in pure condition out of the crude total alkaloids, much being lost in the process of purification. Besides these alkaloids a number of tannins are present in the root.

The Pharmacological action of conessine was studied first by Keidel (1878) who found that this alkaloid depressed the centres for conscious sensation and voluntary movements in the brain. The activity of the vasomotor centre was depressed and the reflexes tended to disappear. The alkaloid also increased the peristaltic movements of the intestines and induced contractions of the bladder. It was concluded that the action of conessine resembles that of morphine. Warnecke (1888) found that the alkaloid was excreted in the urine of dogs as dioxy-conessine. Burn (1914) stated that both conessine and holarrhenine have a similar physiological action. They both produce a considerable narcotic action in the frog but this action is inappreciable in mammals. They also possess a marked anæsthetic action on the cornea. Both the alkaloids produce local necrosis when injected subcutaneously. Both conessine and holarrhenine delay the conduction in the auriculo-ventricular bundle producing inco-ordination and heart-block. The isolated heart of a rabbit comes to a standstill halfway between systole and diastole. In small doses intravenous injections of both alkaloids produce a rise of blood pressure. This rise is still present when the vaso-motor nerves are paralysed, so it must be due to the direct action of the alkaloids on the muscles of the arterioles. Brown (1922) appears to have been the first worker to test the power of conessine, the chief alkaloid of kurchi, as an amœbicide. Working with cultures of pond amœbæ he found conessine to be distinctly lethal and that when it was incorporated with the culture-medium in a strength of 1 in 1,000,000, it inhibited their growth. Experiments with mice showed it to be much less toxic than emetine but subcutaneous administration in medicinal doses, according to him, produced local necrosis. On the other hand, he found that it can be safely given by the mouth in large doses. Henry and Brown

(1923) on testing the tannins of kurchi bark and of ipecacuanha against the free-living ciliate protozoon, *Glaucoma,* found both to be highly toxic to this organism. The alkaloids have little or no effect on the bacilli of the dysentery group.

Pharmacological action.

The pharmacological action of conessine has been fully studied by Chopra and co-workers (1927). It was found to be lethal to *Paramoecium caudatum* in a dilution of 1 in 2,800 of its molecular weight, and in a dilution of M/20,000 in the presence of N/200 NaOH. It killed free-living amœbæ in a dilution of M/1,000 and in a dilution of 1 in 280,000 in the presence of N/200 NaOH. Its action on the vegetative forms of *Entamoeba histolytica* was tested on the dysenteric stools of experimentally infected kittens. In mucus flakes in such stools it paralysed the motile amœbæ, in dilutions of 1 in 280,000 in 8 minutes in the presence of an alkali, and in 18 minutes in the absence of alkali. Emetine in dilutions of 1 in 200,000 was found to be ineffective against the amœbæ, but paralysed them in alkaline medium. Conessine had but little action upon *Trichomonas hominis,* but was markedly lethal to the coprozoic flagellate protozoon, *Bodo caudatus.* Conessine salts can be given subcutaneously and intramuscularly. Contrary to what was reported by Brown, these workers found that no necrosis of the tissues followed its hypodermic injection. Intramuscular and subcutaneous injections of conessine hydrochloride produce less local reaction and pain than emetine.

Gastro-intestinal tract. These alkaloids have an inhibitory action on the activity of the digestive ferments such as ptyalin, pepsin and trypsin. The peristaltic movements of the intestines are inhibited. The alkaloids have no emetic action and do not produce nausea.

Circulation. Small doses, 2 mgm injected intravenously into the saphenous vein of a cat weighing 2 kilo caused a persistent fall of blood pressure, but without any alteration in the intensity or frequency of the heart beat. Perfusion experiments with the isolated heart rarely showed any effect on the frequency or force of the contraction. Doses of 2 to 5 mgm in a cat of 2 kilo showed no alteration in the auricular and ventricular contractions as seen in myocardiographic tracings.

Uterus. The total alkaloids have very little effect on the excised uterus or on the uterus *in situ.*

Excretion. The alkaloids are mainly excreted by the gastrointestinal tract and by the kidneys. They can be detected in the urine soon after intramuscular or oral administration. The excretion goes on for nearly a week after cessation of the treatment.

Local effects of intramuscular or subcutaneous injection. When a 6 per cent solution was injected into the tissues no hæmorrhage or necrosis was observed, but a good deal of œdema occurred at the site of injection. The œdema was most marked in 24 hours and disappeared completely in 48 hours after the injection; hyperæmia and œdema were due to the acidity of the salt of the alkaloids. One to two grains of the total alkaloids give rise to no signs of hæmorrhage or necrosis such as is seen with emetine or quinine.

The action of low-melting point alkaloids has been worked out by Chopra and his co-workers (1933) and is very similar to conessine.

Absence of undesirable effects.

There is no emetic or depressant effect in man when 20 grains of the bismuthous iodide salt of the total alkaloids are given daily for 10 days. The pulse remains normal in frequency, tension and rhythm. There is no alteration in the heart sounds, even in a case of cardiac disease. The drug does not cause irritation of the alimentary canal in the way that emetine does. If it does occur, this is probably due to a co-existing infection with *B. dysenterica* (Flexner or Strong). The drug is, however, exceedingly bitter.

In Amœbiasis

The bark and seeds of *H. antidysenterica* have been used in the treatment of dysenteries of India for many centuries. It appears to be used in the indigenous systems in two forms:—

(1) As kurchi bark, either in the form of a powder or watery extract.

(2) As *Indra Jav,* an emulsion of seeds. Tull Walsh (1891) refers to the use of the bark as being approximately equal in its effects to emetine mercuric iodide. The drug, however, was not tried properly in the treatment of dysentery until lately. Tablets made

from the powdered bark have been put on the market by Burroughs Wellcome & Co., and have been combined with emetine injections. This drug has been used as an adjuvant to emetine in the treatment of chronic amœbic dysentery with excellent results. Different preparations of the drug have been tried in the treatment of amœbic dysentery.

The drug is now used in one of the following forms:—

(1) *Bark.* Sixty grains of the bark in the form of tablets or 1 to 2 drachms of the extract are given orally every day for ten days. The improvement is less rapid than with emetine but seem to be more lasting. The ratio of probable cures to failures by this treatment in a series of 16 cases was 1: 1.

Emetine may be combined with *H. antidysenterica* bark. The latter may be given either in the form of powder or extract together with a course of emetine injections. In a series of 9 cases the ratio of probable cures to failures worked out to be 1: 1.

(2) *Conessine.* One to two grains of conessine are given intramuscularly every day for 10 consecutive days. The drug is well tolerated but the results are not so good as when the powdered bark or the extract is given. Henry and Brown (1923) found the tannins of *H. antidysenterica* bark highly toxic to free-living protozoa and it is possible that the superior action of the powdered bark and the extract is probably due to the presence of these compounds. The ratio of probable cures to failures in a series of 9 patients treated by this method was 1: 2.

(3) *Total alkaloids.* Injections of total alkaloids have been tried in a number of cases. In uncomplicated acute cases of amœbic dysentery, the vegetative forms of entamœbæ disappear rapidly from the stools and as a rule do not reappear. In cases with co-existing bacillary dysentery and in chronic forms of amœbiasis the results are less favourable. Total alkaloids have also been given by the mouth; they do not produce nausea or vomiting.

Kurchi-bismuthous-iodide.

(4) *Kurchi-bismuthous-iodide.* Acton and Chopra (1929) prepared bismuthous iodide of the total alkaloids of kurchi which they called 'kurchi-bismuthous-iodide'. This double salt is insoluble and can reach the large intestine in sufficient concentration to kill the entamœbæ, if large enough doses are given. The drug can be tolerated in doses of 10 grains twice daily, there being no appreciable effect on the heart or blood pressure, no depressant or emetic effect and no irritation of the gastro-intestinal tract such as are observed in the case of emetine.

The writer at first tried the effect of injections of the total alkaloids in 10 cases of amœbic dysentery some of which were acute and others chronic. The drug was given in doses of one grain daily intramuscularly; a marked local reaction was however produced. In this series there was one failure. In another series of 20 cases kurchi-bismuthous-iodide was given by mouth twice daily in doses of 4 grains curing 12 cases. Two required a second course; of these one was cured.

It will thus be seen that in acute cases of amœbic dysentery kurchi alkaloids resemble emetine in their immediate effect on the symptoms as well as in their curative value, in one grain doses. No toxic effects were noticed with injections of two grains daily except flushing of the face immediately after the injection, but this did not last for more than a few minutes. Some patients complained that they could not sleep after injection of such large doses. The injections were however very painful and had to be given up.

Modes of administration.

The cause of failure in treatment both with emetine and the kurchi alkaloids is due to secondary bacterial infection of these ulcers by streptococci and by the dysentery group of bacilli which form acid. These alkaloids are much more powerful when acting in a substrate at a pH of 8 than when acting at a pH of 6. There are three methods of overcoming this acidity of the gut contents. The first method, and one that seems the most obvious, is to administer large doses of alkalies by the mouth, *e. g.*, one to 2 drachms of bismuth carbonate every 2 to 4 hours, sodium bicarbonate or potassium citrate. These drugs have very little effect in reducing the acidity of the large intestine. Clinically this method is useless. The second method is to deal with the organisms that are responsible for the production of this acidity by isolating them, preparing a vaccine, and then immunising these patients against their own organisms. In this way it has been possible to alter the pH of the stool from 6.8 to a pH of 7.35 after three weeks of immunisation. That this method is a sound one is shown by Acton and Chopra (1929); even in failures with emetine, cure can be effected by combined treatment with vaccines and emetine.

More recently Acton and Chopra (1933) by using the bismuthous iodide compound of the total alkaloids of Kurchi in doses of 10 grains (0.6 gm) twice

daily for 10 to 20 days, preceded half an hour before by a mixture containing one drachm of sodium bicarbonate and 40 grains (2.6 gm) of sodium citrate, obtained a proportion of probable cures to failures of 3.16: 1 in a series of 78 cases. This is now the standard method of treatment with kurchi alkaloid. In obstinate and persistent cases, a prolonged course (1 to 3 months) of standardized extract of kurchi,1 to 2 drachms twice daily, with or without *plantago ovata* (Isabgul) is often effective. The extract is extremely bitter to taste and many patients object to it.

The third method is to give the drug in large doses in order to attain a sufficiently high concentration to be effective in an acid substrate. Fortunately, the total alkaloids of kurchi bark are not toxic, so that large doses can be given, and we can also form an insoluble bismuthous iodide compound, which will not begin to act until it reaches the acid substrate in the cæcum and large intestine. This actually does occur, as qualitatively it is found that the kurchi alkaloids appear in the urine of patients taking the bismuthous iodide compound. *Entamœba histolytica* may be living in the tissues or in the lumen of the gut. In the former case it will be in contact with the serum and body fluids at a pH of 7.2, whilst in the gut the contents may reach a high degree of acidity between a pH of 5 and 6.

Discordant results.

The discordant results so far as kurchi-bismuthous-iodide is concerned, are due to:—

1. Some fault in the procedure of extraction of the alkaloids from the bark or preparation of the compound.

2. The bark from which the alkaloids are extracted not being mature, being improperly collected or being imperfectly dried.

Kurchi-bismuthous-iodide was originally prepared in the Calcutta School of Tropical Medicine on the same lines as emetine-bismuth-iodide by the cold extraction process. Its approximate composition is as follows:—Total alkaloids 27 per cent, bismuth 22.85 per cent, and iodine 50.15 per cent. The dose is 10 gr twice daily preceded half an hour by an alkaline mixture. In simple cases a course of 10 days' duration is given; when mixed infection exists 15 to 20 days may be necessary.

Summary.—Two preparations of Kurchi bark are used in the treatment of amœbic dysentery. Kurchi-bismuth-iodide is given in doses of 0.25 gm (4 gr) twice daily for ten consecutive days. In relapsing cases and severe infections, double of this dose may be given for the same period without producing any deleterious effect. The fluid extract of the bark is standardised to contain one half grain (0.032 gm) of the alkaloids in one drachm (4 ccm) of the extract. The extract is given in doses of 1 to 2 drachms 2 or 3 times a day for a period of 10 to 15 days in acute cases and for 4 to 6 weeks in chronic cases. In relapsing dysentery the author has used the extract in doses of 1 to 2 drachms twice daily for months with courses of chiniofon or carbarsone in between, with good results. The disadvantage is that the extract is extremely bitter to taste.

8. Other Drugs in Amœbiasis

Compounds of mercury. The antiseptic action of mercury compounds is well-known and many of them have been tested *in vitro* in the hope of getting a compound which is more parasitotropic and less organotropic, but none of them have been found effective in amœbic dysentery.

Mercurochrome 220 has been tried in the treatment of amœbiasis. For detailed description see the section on mercury.

Kaolin, also known as 'bolus alba', is a native hydrated aluminium silicate purified from sandy matter by elutriation. Extensive deposits are found in China. Deposits of kaolin are present in southern India. It occurs as smooth white or yellowish white powder or in

lumps, insoluble in water, in cold dilute acids, and alkaline hydroxides. It is a powerful adsorbent and has an earthy clay-like taste and odour when moistened. Internally it is used in doses of 3/4 to 1 ounce (8 to 30 gm.) with water, milk or gum acacia emulsion. It is best given on an empty stomach. Kaolin and talc (a purified native hydrous magnesium silicate) have been given as a substitute for bismuth in gastro-intestinal affections; both are insoluble and unaffected by acids. Kaolin along with charcoal, talc, kieselguhr, etc., has a remarkable power of adsorbing toxins and antitoxins. Diphtheria toxins and antitoxins are adsorbed by animal charcoal but not by kaolin, talc, kieselguhr or wood charcoal. Tetanus toxin is adsorbed by all the above agents. In general gram-positive bacteria are adsorbed much more readily than the gram-negative. Colloidal kaolin has been tried intravenously in septicæmic conditions, but it has a direct hæmolytic action on the blood; it also tends to produce allergic shock.

On account of its adsorbing power kaolin has been given by the mouth in the treatment of acute bacillary and amœbic dysentery in doses of 20 to 30 gm stirred in water after a preliminary cleansing of the bowels with calomel. It is particularly useful in cases where a large number of stools have been passed and symptoms of toxæmia are present. It is undoubtedly useful in chronic cases. Asiatic cholera has been treated by kaolin, 400 to 800 gm being dissolved in a litre of water; three ounces or a wineglassful of this suspension is given every half hour till symptoms abate, then every hour or every two hours up to 12 to 15 hours. In a series of 41 cases similarly treated with kaolin no death occurred, while of 24 treated with hypertonic saline 7 died. It is said to have the property of adsorbing bacterial toxins and improving the general condition of the patient. Rectal injections of the same suspension have also been recommended in this disease. The action appears to be entirely mechanical. It is also useful in diphtheria, ptomaine poisoning, bacillary dysentery, etc.

Osmo-kaolin or colloidal kaolin also has been tried. A commercial variety of this is called *collasan*. The dose should be a teaspoonful or more in water, two or three times a day, combined with an aperient if necessary. Colloidal kaolin should contain no organic or inorganic matter and should give no growth of bacteria either ærobically or anærobically. This preparation is a very efficient adsorbent of bacteria and toxins and has been used with advantage in chronic intestinal disorders and for healing ulcerations of the mucous membranes. A useful prescription is, osmo-kaolin 3½ ounce, tragacanth mucilage 1 ounce, peppermint oil 10 minims, water 8½ ounces. A wineglassful of this is given every 6 hours. Osmo-kaolin has also been tried in amœbic and bacillary dysentery as well as in rheumatism and gout. It can be substituted for bismuth carbonate in the combined treatment with emetine and bismuth.

Kaylene is described as activated colloidal aluminium silicate. It adsorbs toxins and is said to act as an intestinal antiseptic. It has been recommended in chronic dysentery, diarrhœa and also for Asiatic cholera.

Bismuth Subnitrate.—Deeks (1914) advocated large doses of bismuth salts by the mouth, and as much as 180 grains of the subnitrate mechanically suspended in a glass of milk or water were given day and night every three hours. Strict dietary was maintained during first ten days and afterwards a non-irritating diet was given. Such large doses are not favoured in these days and toxic effects in form of cyanosis and forcible action of the heart occurred.

Rivanol (2-ethoxy-6: 9 diamino acridine lactate) is an acridine dye and is recommended to be given as an enema in 1 in 2,000 solution, 500 to 800 ccm being injected at body temperature, the patient lying first on the left side and afterwards adopting knee-elbow position. Its action is said to be increased by administration of sodium sulphate by mouth. A 1 in 1,000 solution kills *E. histolytica* in culture in 20 to 24 hours. Experiments on cats infected with *E. histolytica* did not prove definitely its curative effect when given per rectum. It produced spasmodic contractions of the smooth muscle of the gut very similar to those produced by papaverine. Rivanol is given in tablets by the mouth in doses of 30 to 50 mgm daily. Children recieve one tenth of the dose.

Iodoform was recommended in doses of 50 mgm in keratinized capsules; first day one capsule, second day two capsules, third day four capsules, fourth day six capsules and continued for 15 to 22 days, with indefinite results.

Heptyl resorcinol. Faust (1930) offered evidence that heptyl resorcinol is successful clinically in amœbiasis. Of the many compounds only hexyl, heptyl and octyl resorcinol are available. The toxicity of these compounds on oral administration appears to increase with the increase in size of the alkyl radicle. Octyl resorcinol may be definitely ruled out because of its local irritating effects. In natural *Balantidium coli* infestations in guinea-

pigs heptyl resorcinol was found to be definitely curative, but in doses approaching the MLD hexyl resorcinol is less toxic than heptyl, has a stronger antiseptic action and is worthy of trial.

9. Summary of Treatment of Amœbiasis

Every person harboring *E. histolytica* has a lesion either in the intestine or elsewhere. There is no such thing as a healthy carrier. Treatment should, therefore, be given in every case in which *E. histolytica* is discovered and if possible the disease should be eradicated.

Symptomless carriers.

It is most important that patients without symptoms or with mild symptoms should be thoroughly examined both from therapeutic and prophylactic points of view. For such cases the following treatment is advised:—

Scheme of treatment for Entamoeba Histolytica carriers without co-existing dysentery—

Days 1-10—Carbarsone 0.25 gram by mouth twice daily.

,, 20-25—Emetine hydrochloride 0.065 gram (one grain) daily, intramuscularly.

,, 20-30—Carbarsone 0.5 gram by mouth twice daly.

If stools positive on the 37th., 38th., or 39th. day, or on later clearance tests, patient is to be treated on the lines as described under chronic Amœbic Dysentery.

The following scheme of treatment is recommended in acute or subacute amœbic dysentery:—

Treatment of acute or subacute Amoebic Dysentery.

Days 1- 6—Emetine hydrochloride one grain daily, intramuscularly.

,, 1-10—Carbarsone 0.25 gram by mouth three times daily.

,, 10-24—Diodoquin 0.63 gram by mouth three times daily if stools negative on the 8th., 9th., and 10th. days.

If stools positive on the 10th. day or symptoms persist:

Days 10-20—Vioform 0.25 gram by mouth three times daily and chiniofon retention enemata, 2½ per cent, every other day, 250 cc. to be retained for eight hours.

,, 18-20—Emetine Hydrochloride 0.65 gm. (1 grain) daily, intramuscularly.

NOTE:—In the absence of adequate supply the following drugs in their respective dosages may be considered interchangeable:—Chiniofon one gram three times a day, vioform 0.25 gram three times a day, diodoquin 0.63 gram.

Treatment of Chronic Amoebic Dysentery.

If the patient is seen during an acute exacerbation, the same treatment as in case of an acute attack is given. During the quiescent period, the patient should be given a course of chiniofon, diodoquin, vioform, or carbarsone (15-20 days). If after the course, the stools still contain entamœba in vegetative or cystic forms, the course should be repeated. In case of carbarsone the course should not be repeated till three months after the previous course, but during this period courses of chiniofon or allied compounds may be given.

In very chronic cases who have repeated relapses, the eradication of the disease is very difficult and treatment may have to be continued for months, with suitable intervals. Combined treatment with emetine-bismuth-iodide and chiniofon in form of high retention enemas has been recommended by some authorities.

The treatment in these cases should be controlled with repeated stool and sigmoidoscopic examinations. If either motile or cystic forms of entamœba, or ulceration, is present the course should be repeated.

In these cases no one method of treatment is certain. If one remedy fails, another should be employed. The writer has tried courses of kurchi-bismuth-iodide combined with carbarsone or chiniofon with success. In persisting cases repeated course of carbarsone or chiniofon or diodoquin and prolonged use of kurchi extract afterwards in doses of one to two drams (4 ccm) twice daily for a period of three months at a time, has produced useful results.

The following plan of treatment is recommended in these cases:—

Days 1- 6—Emetine hydrochloride gr. I subcutaneous injection nightly.
„ 5-14—Chiniofonum (Quinoxyl, yatren) retention enemata 2½ per cent or 4 per cent each morning, 250 cc to be retained for eight hrs.
„ 7-12—Emetine bismuth iodide grs. ii or iii by mouth on an empty stomach each night.
„ 13-25—Tabs. amibiarson 0.25 gm. twice daily after food.
„ 20-25—Emetine bismuth iodide grs. ii or iii as before.
„ 29-31—Three stools to be examined for clearance.
„ 29-31—Sigmoidoscopy.

A further examination of stool should be made during the period 46-53 day (*i.e.* 3 weeks after treatment) and if the bowel mucosa at the first sigmoidoscopy appeared unhealthy. this examination should be repeated at this stage.

Scheme of treatment for Amoebic Hepatitis and Liver Abscess:—

Amoebic Hepatitis and Liver absess.

Days 1- 9—Emetine hydrochloride 0.065 gram (one grain) daily intramuscularly.
„ 1-14—Chiniofon in doses of one gram by mouth three times daily.
„ 13-15 } If clinical or laboratory evidence indicates advisability of
„ 30-35 } further emetine therapy, inject 0.065 gram (one grain) Emetine hydrochloride daily intramuscularly.

Carbarsone is contraindicated in the presence of hepatitis.

Supplementary therapy—Specific therapy should be supplemented with injections of liver extract, administration of vitamins, especially, B and C, plasma, blood, or glucose transfusions, when necessary.

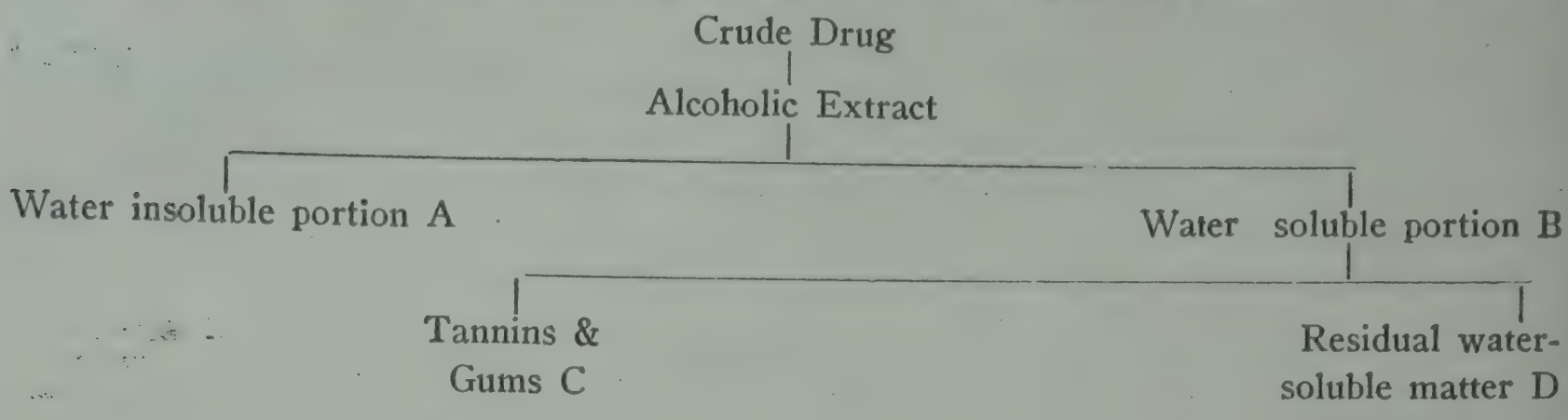
10. Palliative Remedies in Amœbiasis

As a considerable residuum of cases of amœbic dysentery is left uncured. Henry and Brown (1924) examined a number of reputed remedies against this disease.

Monsonia ovata and *Rhyncosia adenodes* are used in Abyssinia and Malaya respectively. These drugs have been examined chemically without showing any active constituent to which their amœbicidal action could be attributed. *M. ovala* which is one of these remedies and a substance called *entericin* was isolated but entericin is an ill-defined substance. From the two species of Brucea mentioned above, amorphous bitters have been isolated, but trials on free-living protozoa show them to be quite inactive either alone or in the presence of alkali.

The bark of *R. adenodes* was examined by Henry and Brown, but no active substance other than tannins was found. These investigators tried to combine biological and chemical methods with the hope of selecting from the large number of such available drugs any which seemed promising enough for detailed examination. The finely ground drug was exhausted with boiling alcohol and the extract concentrated *in vacuo*; the thick syrup obtained was diluted with water to precipitate resinous and fatty matter and this gave

preparation A. The liquor was further concentrated *in vacuo* to remove all alcohol, and this was B. This was treated with lead acetate to remove all tannins and this preparation was C and the residue after recovering the precipitate and removing excess of lead was D.



The strength of this final product was adjusted so that 1 ccm represented a certain quantity of the drug. All four portions were then tested on glaucoma, an actively motile ciliate occurring in hay infusions. A and D proved non-toxic to this organism and B and C were toxic. The activity was found to be due to one of the components of C and on testing it was found to be the tannin fraction.

It is interesting to note that the action of many of the drugs used against non-specified dysenteries in India and elsewhere is due to their astringent effects and not to the toxic effects of any of their constituents, on the parasites. The chemistry of tannins is still not very advanced. Only a few tannins are obtained in pure condition, *e.g.,* gallotannin (from galls) which can be obtained commercially in crystalline form; ellagic acid, chebulinic acid (from myrobalans), catechin (from Gambier catechu) can also be obtained in more or less pure form. Some of the tannins are readily soluble in cold water and these are in most cases toxic to glaucoma; other soluble in boiling water are nontoxic. The crystalline and sparingly soluble tannins have little or no action on glaucoma. The toxicity of tannins appears to be the inherent property of these compounds, but their action is not very powerful. They kill glaucoma in dilutions of 1 in 1,000 to 1 in 2,000, while the alkaloid conessine from *H. antidysenterica* kills in 1 in 120,000 or more. In view of these facts it appears to be unlikely that any of these tannins or the vegetable drugs which contain them will prove to be of any great utility in resistant forms of amœbiasis.

(1) Plantago Ovata (Ispaghula)

This is known in the vernacular as *Ispaghula* or *Isabgul.* It is a Persian herb which also grows in the Punjab, Sind and the United Provinces. The seeds which are commonly used are imported from Persia into Bombay in large quantities. In the Tibbi books the seeds are found described under the name of *Bazr-i-katuna.* They are boat-shaped and generally pinkish grey, but the colour may vary, some being brown while others are nearly white with a pinkish tinge; the latter are preferred. Isabgul is a well-known and popular remedy in chronic dysentery in India. So commonly is it used in this condition even by Western practitioners that it has found its way into the B. P. C. When steeped in water the seeds swell up giving out large quantities of a sticky, bland mucilaginous substance with no taste or odour. The seeds of several other species of the same genus exhibit similar properties; *Plantago amplexicaulis* grows in many parts of India and furnishes the brown variety of seed; *P. major* (Luhuriya) grows in the Himalayas; other species of plantago are *P. psyllium* (which is practically the same as *P. major*), *P. brachyphylla* and *P. lanceolata.*

The pericarp which contains all the mucilaginous matter is removed from the seed and is sometimes prescribed instead of the whole seeds by indigenous practitioners. It is sold in the bazar under the name of "Bhusi" and it is administered in doses of 2 to 3 dessert spoonfuls 2 or 3 times a day. When the seeds are roasted they are said to have a more astringent effect.

Clinical Composition.

The seeds were examined by Henry and Brown (1923). The chief constituent is the mucilage which is contained in the cells of the epidermis. After excluding the mucilage they could find no active principles toxic to protozoa except tannins. The author found a glucoside named *aucubin* in small quantities.

Pharmacological uction.

The jelly-like substance occurring in the seeds is acted on by the digestive enzymes to a very slight extent and therefore most of it passes through the small intestine unchanged. It is also not acted on by the intestinal bacteria such as *B. coli, B. shiga, B. flexner.* The seeds coat the surface of the ulcer and also adsorb the toxins present in the gut.

The seeds on account of their emollient and demulcent effects, are commonly used in India as a remedy in chronic diarrhœa and dysentery. They are thoroughly cleaned from sand and grit with which they are always mixed. Two to 4 heaped dessertspoonfuls prepared in one of the following ways may be taken: (1) With a little sugar and shaken up in a cupful of water. (2) The seeds are mixed with water and allowed to stand for 15 to 20 minutes till all the mucilage comes out. The swollen mass is then swallowed. (3) A mucilaginous decoction made by boiling one to two tablespoonfuls of the seeds in 2 pints of water till the volume is reduced to half. This is given in divided doses, and is preferred especially in acute dysentery. *Therapeutic uses.*

The first method is preferable as it allows the seeds to mix with the intestinal contents thoroughly so that they spread over the whole of the surface of the mucous membrane evenly; whereas if the mucilage is allowed to form before administration it forms a sticky mass which is not evenly distributed and much of the demulcent action is lost. The drug has no amœbicidal effects and its action appears to be entirely mechanical. The irritated or ulcerated surfaces of the intestinal mucosa are soothed by the demulcent action of the mucilage, which is not acted on by the intestinal juices. It covers the surface of these ulcers and in this way protects them from further irritation. The drug is tasteless and is equally suitable for children and adults.

There is little literature on the use of this drug in dysentery, but it has been unanimously acclaimed as having a remarkably beneficial action in chronic dysentery. The author has given extensive trials to the seeds in chronic diarrhœa and dysentery with excellent results and strongly recommends this drug in chronic irritative diarrhœa and dysentery of both amœbic and bacillary origin. In chronic spastic constipation the drug produces a laxative action. It should be remembered, however, that unless sufficiently large quantities of the seeds are taken no action is produced. One or two heaped tablespoonfuls may be given at a time once or twice daily. It has a laxative action similar to agar.

A number of proprietary preparations composed of the mucilage containing principle of the seeds have been recently put on the market.

(2) Aegle Marmelos (Bael Fruit)

It is commonly known as 'bæl' tree and has many vernacular names, *Billwaphal* or *Sriphal* in Sanskrit, *Bael* in Hindi and Bengali.

The tree is indigenous and is cultivated all over India. In Hindu medicine the root bark is used in the form of a decoction as a remedy in hypochondriasis, melancholia, intermittent fever and palpitation of the heart. The leaves are made into a poultice and applied to the inflamed parts. The medicinal properties of the fruit have long been known to Hindu physicians. Two kinds of the fruit are found in the bazar—a small and wild variety, and a large cultivated variety. The full grown fruit of either variety, when it just begins to ripen, is best for medicinal purposes. It is used in two forms:— *Fruit.*

(1) *The unripe or half-ripe fruit.* This is regarded as an astringent, digestive, and stomachic and is an excellent remedy in irritation of the alimentary canal owing to the presence of tannins or mucilaginous substances. It is sometimes used in combination with opium by Ayurvedic practitioners.

(2) *The ripe fruit.* This is sweet and is considered as aromatic and laxative. The dried pulp is pale yellow or flesh-coloured and when mixed with water yields a pleasant orange coloured 'sherbet' which has slightly laxative properties.

Both unripe and ripe fruits contain tannin in small quantities. The dry pulp moistened with cold water yields a red liquid chiefly containing mucilage and probably 'pectin', which separates if the liquid is concentrated by evaporation. As far as is known the principal constituent of bael fruit is mucilage. Henry and Brown (1923) found only small quantities of tannins present in the ripe fruit, some of which were toxic to glaucoma. More recently Dutt & Dikshit (1930) found in the pulp, besides the usual constituents, a body known as *marmelosin.*

The fruit is said to have an astringent action and this property is not lost by drying, although fresh fruit is preferable. It is said to possess a remarkable efficacy against dysentery and diarrhœa; so commonly was it used by Western practitioners in India in old *Therapeutic uses.*

days that it was included in the British Pharmacopœia in the middle of last century. It was believed to be an invaluable remedy in obstinate cases of chronic diarrhœa and dysentery unattended with fever, and was given in the form of a powder or as a confection. There is very little literature available regarding the effects of 'ba el' in amœbic dysentery. During, recent years the drug has not been tried on a large scale and controlled by proper examination of stools. It has little or no effect in acute dysentery, though the powder is said by some to produce good effects in this condition. The first noticeable effect is that blood rapidly disappears and the stools assume a more fæculent form. The main action of the drug in chronic dysentery is due to its demulcent properties and also to a certain extent to the tannins present in it. The drug is worthy of trial in chronic amœbiasis where other remedies have failed.

Preparations.

(1) *Extract of bael.* This is made from fresh unripe fruit. Dose ½ to 1 drachm 2 to 3 times a day.

(2) *Liquid extract of bael.* This is prepared from dried slices of unripe fruit. Dose ½ to 1 drachm several times a day. It is said to be much less effective than the fresh extract.

(3) *The powdered dried pulp.* This is said to keep well in airtight bottles. Dose ½ to 2 drachms in chronic dysentery.

Decoction of the dried bael fruit and syrup made with fresh or dried fruit are also used.

(3) Acorus Calamus

It is commonly known as 'sweet flag', in English, 'Bach' in Hindi and Bengali. It is a semi-aquatic perennial herb with an indefinitely branched rhizome. It is really a native of Europe and North America, but is cultivated in damp marshy places in India at an altitude of 3,000 to 6,000 feet; it has established itself on the edges of lakes and streams. The long creeping horizontal rhizome is collected in the autumn, cut into pieces and dried.

The dried rhizome yields 1.5 to 3.5 per cent of a neutral yellow aromatic essential oil having an agreeable odour. The chief constituent of this oil is asaryl aldehyde. There is also a bitter glucoside named acorin and certain other substances, such as eugenol, asarone, pinene and camphene are present. Henry and Brown (1923) found an essential oil which was toxic to glaucoma but no other active principle except tannins.

Therapeutic uses.

The rhizome is emetic, nauseant, anti-spasmodic and carminative. In doses of 35 to 40 grains it produces a violent and persistent emesis; it has an expectorant action and is used as a remedy for asthma. The drug is a very old remedy for chronic diarrhœa and forms part of a number of mixtures in the Hindu medicine. Evers (1875) tried it in chronic dysentery with good results. It has been discarded in recent years, and any effect it produces is due to the presence of tannins.

(4) Helicteres Isora

It is commonly known as the East Indian screw tree belonging to the Sterculiaceæ family. In Hindi it is known as *Marar* or *Mararphali* (or the pod used in dysentery), in Guzrati it is known as *Mriga shingha* or deer's horn. It is a tall shrub, or a small tree resembling the common hazel. It has bright red and showy flowers which appear in the rainy season. The plant grows in Western India and in Kashmir. According to Ainslie it is used by Hindu physicians as a remedy for offensive sores inside the ear. It also forms part of prescriptions used for the cure of griping in the bowels and flatulence especially in children. The pods have demulcent and slightly astringent properties.

The writer analysed the pods, but no active principles with the exception of tannins and the demulcent substances could be discovered.

The pods are used in some parts of India in the treatment of chronic dysentery. In some patients the symptoms are considerably ameliorated.

(5) Castela Nicholsoni

This plant is known as *Chaparo amargosa* in Mexico and was used by Mexicans in the treatment of dysentery. It is a semi-rubiaceous plant and its active principle is a glucoside called *castelin* and a bitter principle called *castelamarin*. Castelin crystallizes in long white needles and dissolves in 85 parts of water and is also soluble in alcohol. It is dextro-rotatory and is readily hydrolysed by dilute acids, yielding *castelagenin*.

The 'chaparo' (any part of the plant, root, leaves, bark), is powdered and measured in heaped teaspoonfuls. Five teaspoonfuls are given daily, *i.e.,* three as drinks, each prepared by boiling a teaspoonful of the powder in 8 oz (240 ccm) of water for a quarter of an hour and two for enemata similarly prepared with 12 oz (360 ccm) of water. The decoction is cooled and strained before administration. The enema is given by a long rubber catheter, passed as high as possible, with the patient in the knee-elbow position. The drinks are given half an hour before breakfast, lunch, dinner and supper and enemata at 10 a. m. and 6 p. m. The treatment is continued for 10 consecutive days. The decoction has a very bitter taste. Some cases, apparently incurable with emetine, appear to have been benefited by 'chaparo'.

(6) Simaruba Bark

Simaruba bark is obtained from various species of Simaruba, *S. officinalis, S. amara, S. glauca,* etc. It was brought to Paris in 1713 from Guiana, where it was said to have been used with success by the natives in the treatment of dysentery. It soon gained a reputation in Europe and was imported in large quantities. The active principle of the bark is a crystalline bitter substance possessing neither alkaloidal nor glucosidal properties. It has been used in amœbic dysentery with some success in a similar manner as 'chaparo', a handful of bark being cut up and a decoction prepared in the same way. It does not produce any marked therapeutic effects in this disease and is now seldom employed.

Uzara

Uzara is another African plant belonging to the N. O. *Asclepiadaceae.* It is a proprietary article recommended by certain German workers, but is of doubtful efficacy. It is given in form of a tablet. The alcoholic extract is known as *Panzaron.*

Ya Tan Tzu

Ya Tan Tzu is the chinese name for the seed of *Brucea* Sp: called by some *Brucea Sumatrana,* by others *B. javanica* and *B. amarissima.* It has been found to contain an alkaloid "brucamarine" and a bitter glucoside 'Kosamine;" the seeds contain also a small quantity of hydrolytic enzyme, 1.8 per cent of tannin, 20 per cent of a fatty oil, and two bitter principles.

Two per cent cold infusion of powdered seed, freed from the oil killed amœba instantly in vitro. The ethereal extract has also an amœbicidal action, but the expressed oil has not. For the treatment of dysentery, acute and chronic, it is given in doses of 20-50 seeds (shells removed). If amœbæ are still seen after 2 or 3 days, the dose is increased. The treatment appears to be effective. The amœbæ disappeared from the stools in an average of 2.6 days. The drug is easy to administer, only a few doses are needed and it is cheap.

DIARRHOEAS OF PROTOZOAL ORIGIN

11. Balantidiasis

Balantidiasis is also known as Balantidial dysentery or Ciliate dysentery.

It has been shown that forms of diarrhœa or dysentery caused by a ciliate protozoan *Balantidium coli* may be met with in man. This ciliate is common in pigs and monkeys and may produce in them a fatal form of dysentery. Human cases have occured in those who are intimately associated with pigs by faecal contamination of food and hands. Cases have been recorded in many of the countries all over the world and fatalities have occured. The parasite penetrates into the intestinal wall in the same way as *E. histolytica* and is found in the blood vessels of the mucosa and submucosa. There is at first hyperaemia of the wall, punctiform hæmorrhages, follicular swelling and overproduction of mucus; the ulcers formed have irregular undermined edges. The parasite penetrates the wall and invades the gland ducts, dissolving the muscularis mucosae by cytolysis. The cysts of this parasite are infective and are viable in the faeces for weeks in moist and shade; they are rapidly killed by drying and sunlight.

Symptoms.

The symptoms produced are the same as those of amœbic dysentery though milder. There is a low grade diarrhœa, loss of appetite, colicky pains, slight nausea abdominal tenderness and distended colon. Eight to fifteen stools may be passed in a day and the colon is tender to touch. The stools are fluid, alkaline in reaction and contain undigested food and mucus and are often stained green. There may be cachexia and anæmia.

Treatment.

Many drugs, such as thymol (same dosage as for ankylostomiasis), calomel, carbolic acid, extract of filix mas, methylene blue, yatren, santonine, oil of chenopodium, stovarsol, etc., have been tried. Enemata of iodine solution, (1 in 10,000), methylene blue, silver nitrate and tannin (1 in 1,000 with 10 to 15 drops of tincture of opium) have been used. Emetine in usual doses, carbarsone acetarsone, carbon tetrachloride have been tried but are not effective. There is no specific treatment but chiniofon in doses given in amœbiasis has given good results.

Giardiasis

Three species of intestinal flagellates occur: *Giardia lamblia, chilomastix mesnili* and *trichomonas.*

12. Giardiasis

Introduction.

Giardiasis also known as Lambliasis is an infection with a protozoan flagellate inhabiting the gut of man and *Giardia intestinalis* has the best claim to be regarded as pathogenic (Manson-Bahr). There is however, still considerable difference of opinion with regard to this. Children appear to be more commonly affected than adults. Flagellates of this genus are found in mammals, reptiles and *G. muris* is closely allied to the species occurring in man. According to some authorities the number of parasites in the gut depend on the diet which is being taken; they flourish on carbohydrates and disappear from the intestines when patients are put entirely on meat diet. The parasite has been found in the duodenum but there is no reason to suppose that it produces disease of the biliary passages. though it has been found in the gall bladder and in the bile.

Effect of diet.

Pathogenicity.

Giardia infection occurs in India, China, Ceylon, South and East Africa, Brazil and other countries. The following arguments are brought in favour of its being pathogenic:—

(*a*) It is found in large numbers and in an active stage when the stools are liquid and diarrhoeic. (*b*) In the early stages, symptoms of gastro-enteritis are present while in chronic stages the stools have characteristic features and large number of cysts are present. (*c*) Mucus passed contains these parasites. (*d*) In children persistent diarrhœa, enlargement of the abdomen and retardation of growth occurs. (*e*) Mucus in the stools is produced from that part of the gut in which this flagellate is most abundant and is the result of irritation. (*f*) Specific therapy gives relief of symptoms.

It is quite possible that certain individuals are more susceptible than others (Wenyon). The patients have recurrent history of dysenteric attacks and soon pass large pale stools, as if suffering from sprue. On the other hand it has been said that a large number of people who have numerous cysts in their stools do not show any symptoms or effects of this infection.

Symptoms.

Duration of symptoms may be from a few months to as many as ten years. There is initial diarrhœa of a lienteric type followed by chronic intestinal disturbances. Flatulence is present and acute exacerbations with diarrhœa and passage of bile-stained mucus are frequent. Lassitude, anorexia, alternate diarrhœa and constipation, abdominal pain and tenderness occur. Stools vary from 2 to 8 per day, with pale or bright yellow colour. The stools are urgent and sometime described as explosive.

Examination with sigmoidoscope shows no changes.

Treatment.

A large number of drugs have been tried. Many drugs including emetine-bismuth-iodide have failed to eradicate the flagellates. Large doses of sodium bicarbonate followed by calomel, magnesium sulphate, thymol, EBI. and high lavage of the colon produce no permanent effects. Large doses of carbonate of bismuth, such as 30 gm given in the morning on an empty stomach for eight days and repeated after two days are said to cause disappearance of the parasite. Intravenous injections of the arsenical compound stovarsol, and yatren (2.5 per cent solution) enema for 10-12 days, showed some amelioration of symptoms but the parasite did not disappear permanently. Mechanical lavage of the bowels with large quantities of fluid produced permanent disappearance. Manson-Bahr alleviated all symptoms by putting patients on a meat diet; the results have been also satisfactory in children.

Atebrine and mepacrine have been used in the treatment of lambliasis in the same doses as in malaria (0.1 gm or 1½ gr thrice daily for seven days); the author has tried this treatment with success in a number of cases. One course of mepacrine suffices in most cases and in a few cases it may be necessary to repeat the course once or twice; even after this, the infection may not be eradicated, though the number of parasites decrease in the stools and clinical symptoms disappear. The infection responds to both oral and sub-cutaneous administration, but the oral route is to be preferred. A butyl acridine derivative, 2-chloro--methoxy-5 (8-diethyl aninobuty) aminoacridine, given by the mouth in doses of 30 mgm for adults and 15 mgm for children, three times a day for five days also produce disappearance of giardia in the stool.

The concensus of opinion is that atebrine is the drug of choice and in majority of cases eradicates infection, as shown by studies of stools after gall bladder drainage by administration of magnesium sulphate.

13. Flagellate Diarrhœa

Introductory.

The question of harmlessness or otherwise of all species of flagellates present in the intestines has been much debated but no solution has yet been found. It has not even been settled whether any of the flagellates, *e.g., Trichomonas* is capable of invading the tissues or of ingesting red blood-corpuscles. It is not known whether they are the cause or effect when they occur associated with diarrhœa. Their presence in large numbers may be due to the fact that they thrive in fluid medium and especially so when the contents have been rendered abnormal by infections, such as bacillary dysentery when they get the suitable medium for multiplying. When, however, they are found in chronic diarrhœa with nothing else to account for the condition, it becomes difficult to say that they are not the causative factor. The term flagellate diarrhœa has, however, come to stay. When flagellates are present in the stools there is always fæcal contamination of food on account of defective local sanitation. A high degree of flagellates indicate exposure to abnormal intestinal infection. This probably accounts for the presence in large numbers of *Trichomonas* and *Chilomastix* in the stool of patients suffering from chronic dysentery and sprue and it should suggest some primary cause of diarrhœa, such as amœbic dysentery. In a large number of cases where flagellates are present, no symptoms of disease, however, are present and the relationship of the organism to the host may be one of communalism. They occur in the mouth and vagina and male urethra.

Pathogenicity.

Others, however, believe that these flagellates are pathogenic and that they can produce diarrhœal conditions of their own and that they disappear after treatment and colonic lavage. The active forms disappear and the cysts appear in the stools. A condition of neurosthenia has been described accompanying these infections due to irritation of the intestinal mucosa and absorption of toxic products derived from the organism. These

Symptoms.

stimuli transmitted through the sympathetic, cause loss of energy resulting in neuresthenic vasomotor symptoms, such as lassitude, irritability, which are a part of this complex. Neuresthenic symptoms, however, occurs with amœbic dysentery and other forms of bowel diseases. Wenyon has found the organism in the lumen of the gland in sections of the intestines actually invading the gland cells and distributed in the connective tissue. *Trichomonas* have also been seen ingesting red blood cells. In the guineapig an allied species occurs which definitely causes ulceration of the intestinal mucous membrane. Others believe them to be secondary invaders and say that the bowel damaged by dysenteric toxin take considerable time to recover and a heavy infection with flagellates probably act as irritating agents; they should, therefore, be eliminated.

Treatment. There is no specific treatment, though the organisms disappear after vigorous lavage with a 2 per cent sodium bicarbonate solution. Stovarsol in 4 gr doses for 8 to 10 days is said to exert a specific action on *Trichomonas intestinalis.*

Trichomonas vaginalis is easily implanted on a negative virgin vagina from the urethra of the male. Attacks of typical dysentery are said to be caused by heavy giardia infection of the colon and such symptoms are more common in children. When the small intestines are affected there is great tendency to chronicity and periodicity of symptoms and presence of giardia in the stools. When giardia disappear from the stools cysts appear. It has been said that this infection may produce any combination of abdominal symptoms leading even to a surgical operation; the suspicion of tuberculosis has also occured. Giardiasis may occur in association with steotorrhoea, microcytic anæmia and achlorhydria suggestive of sprue or para-sprue.

Treatment. Excellent results have been reported from vaginal insufflation with sulphathiazole or sulphadiazine powder in the treatment of Trichomonas infection. A powder containing sulphathiazole 1 part and betalactose 3 parts was used. Eight gm of the mixture was insufflated daily for four treatments. Flagellates lost their motility in ten to fifteen hours. Blood sulphathiazole levels were not raised to dangerous levels, which is quite understandable, as 2 gm of the drug daily would not be a large dose.

14. Intestinal Coccidiosis

Species. Three species of coccidia occur in cystic stage in the fæces of man. *Eimeria clupearum* and *E. sardinae* which are parasitic on the genitalia of herrings and sardines and which pass through the intestinal canal undamaged. *Isospora hominis* is parasitic in man and accidental infection with this organism produces diarrhœa with typical cysts. The fæces are liquid, brownish yellow and contained numerous Charcot-Leyden crystals. There is a large amount of undigested matter in the stool, but there was no pus or blood. The oocyst persists for 36 days and then vanishes and the patient recovers. Signs and symptoms which were not very prominent were diarrhœa, abdominal discomfort, flatulence and loss of weight.

Isospora hominis also known as *I. belli* is probably a parasite of epithelial cells of the small intestine. The oocysts vary in length from 25 to 33 u and one half as broad; when passed fresh they are transparent and colourless in human fæces. The infection is world-wide and it is probable that in man it completes its schizogenic development in the mucous membrane of the intestinal villi. The symptoms produced are those of sub-acute dysentery and the fæces are light coloured and contain large quantities of undigested matter and excess of fat.

Treatment. This consists of giving bismuth salicylate and charcoal three times daily.

The disease is of short duration and limiting in character and often no treatment is necessary.

CHAPTER II

TRYPANOSOMIASIS

AFRICAN TRYPANOSOMIASIS—CHEMOTHERAPY OF TRYPANOSOMIASIS: ANTRYPOL (BAYER 205, GERMANIN); OTHER DRUGS: DIAMIDINO STILBENE, ANTIMONY COMPOUNDS, ARSENICALS—SUMMARY —SOUTH AMERICAN TRYPANOSOMIASIS.

Trypanosomes infecting animals.

Important trypanosoma infecting animals are:—*T. brucei* causes fatal disease in horses and cattle. *T. evansi* produces disease in horses in India called 'surra' and is believed to be transmitted by the biting of flies *stomoxys*. *T. equinum* causes fatal disease in horses in South America. *T. equiperdum* and *T. evansi* infect horses in many parts of the world. *T. congolense* (*T. dimorphon*) causes disease of cattle, sheep, goats, pigs and dogs in Africa and game may serve as reservoir. *T. vivax* is an active species occurring in cattle, sheep and goat. *T. lewisi* occurs in rats.

1. African Trypanosomiasis

The African trypanosomiasis is also called sleeping sickness or Negro lethargy.

Vector and distribution.

It is a protozoon infection caused by a flagellate named *T. gambiense* (*Var. T. rhodesiense*) which is transmitted by species of tsetse flies of the genus *Glossina* especially *G. palpalis, G. swynnertoni, G. tachinoides* and *G. morsitans;* also by other blood-sucking Diptera *e.g., stomoxys*. The disease is characterised by weakness, wasting and protracted lethargy. The trypanosomes are blood parasites in man and animals and give rise to fever, adenitis, rash and eventually invasion of the central nervous system causing meningo-encephalitis.

Distribution corresponds with that of tsetse fly. *G. tachinoides. T. gambiense* infection occurs in West Africa, Sierra Leona, Gold Coast, Nigeria. *T. rhodesiense* occurs in Nyasaland, Rhodesia, Tanganyka, Portugese East Africa, etc; it is carried by *G. morsitans.* This disease has dominated over one-fourth of the continent of Africa and more than a million cases are treated every year.

T. gambiense, Var. rhodesiense and T. cruzi infect man, the latter causing American trypanosomiasis. *T. gambiense* occurs in the blood, lymphatic system, and cerebrospinal fluid of man and domestic animals. The infection is transmitted by tsetse flies, but human trypansomes may also be transmitted by coitus, and disease in horses known as dourme is transmitted in this way.

Recent evidence has shown that human beings are the most important source of infection for the vector. In other regions wild game may serve as a reservoir of infection, particularly some species of antelope, *e.g.,* bush buck, water buck, etc; domestic animals also act as reservoir, (nagana due to *T. brucei*). Instances of human beings infected with trypanosomiasis and apparently in good health have been reported, and such persons may act as reservoir.

After ingestion of blood by the fly, the parasites multiply in the mid and posterior gut; between 8th and 18th days, slender forms appear and pass into the proventriculus and into the salivary glands and ducts; here further development occurs into the crithidal forms which attach themselves to the gland cells; from these are developed 'metacyclic' trypanosomes which pass down the salivary ducts and from here enter the bite wound.

The fly after imbibing blood containing trypanosomes, becomes infective in 18 to 34 days, in rare cases in 53 days. It then remains infective for 188 days, *i.e.,* for the rest of its life. Direct mechanical transmission by blood on the proboscis of glossina also occurs and may be responsible for some epidemics.

Pathology.

The tissues chiefly affected are the lymphatic glands and the central nervous system. There is proliferation of lymphocytes and retculo-endothelium. The fever in early stages is followed by meningo-encephalitis and meningo-myelitis. The vessels of the brain and cord are compressed resulting in malnutrition, cerebral changes, and the desire to sleep. The trypanosomes are distributed in the brain in an irregular manner. There is adenitis of the neck, groin and other glands. The cerebro-spinal fluid is turbid and the dura mater

thickened and adherent; the spinal cord shows congestion and hæmorrhage. There may be ascites and the pericardial fluid is increased; the spleen is often enlarged.

The cerebro-spinal fluid in early cases may be normal; it shows trypanosomes after about 3 months (on centrifugalising) and always after one year after infection. There is increased pressure and proteins are present; cell count may go to 1,000 per ccm (lymphocytes, mononuclears, eosinophils); in unfavourable cases the mulberry or muriform cells are present. Blood shows definite anæmia and decrease of hæmoglobin.

Symptoms.

Two stages of the disease have been described, febrile or glandular and cerebral.

Clinical aspect.

Stage 1.—Incubation period may be 7 to 21 days from the time of bite to appearance of trypanosomes in the blood. There is characteristic local reaction at the site of the bite and red discoloured nodule or a furuncle about an inch in circumference appears surrounded by œdema—*Trypanosomal chancre*. After 14 to 21 days or longer rigor and fever appear; spleen and liver may enlarge. The temperature approaches normal in morning and 103° to 104° or higher in the evening.

Erythematous eruption consisting of pinkish patches irregular in position and outline, round or oval in shape with a clear centre appear on the trunk and thighs. The skin is dry and pruritis is often present. Glands enlarge in the posterior triangle of the neck at first soft and elastic later hard and fibrous; on puncture they show trypanosomes. Karendels sign, *i.e.*, slightest pressure feels like a bruise and is very painful; a puncture with a needle feels like a hot poker and an accidental knock gives excruciating pain. There is asthenia, anæmia, weakness, wasting, conjunctivitis and keratitis. Low irregular pyrexia may go on for months with temperature of 99° to 100°F in the evening or may end spontaneously or after treatment.

Cases are clinically divided into 3 groups:—

Cases are clinically divided into 3 groups:—(1) those in good health with trypanosomes in blood and with some fever; they are dangerous fertile source of infection to others. (2) those (75 per cent) showing definite clinical symptoms who have a tendency to go to sleep in the day time; they have enlarged lymphatic glands which on puncture show trypanosomes. (3) advanced cases (13 per cent) which show high mortality. Their speech and mentality is affected and they are in a stupurous condition.

The cerebral or meningo-encephalitic stage or sleeping sickness lasts from 4 to 8 months, rarely 3 years. The first signs are tremors of the tongue, fingers, hands, arms and legs; headaches, delusion and mania may be present; œdema may appear in any part of the body. A peculiar shuffling gait and difficulty in raising feet from the ground is present; the deep reflexes are increased at first, then lost; speech is slow, tremulous like that of a sleepy and tired person; Rombergs sign may be present; later rigidity of neck and legs appears, pulse rate is 90 to 140 per minute, and out of proportion to temperature.

The patient shows tendency to sleep, or a lethargic condition. As the disease progresses, there may be emaciation, low tension pulse, dribbling of saliva, incontinence of urine and fæces, bed sores, subnormal temperature, ending in coma and death. Complications are pneumonia, Cheyne-stokes breathing, dysentery, œdema of glottis and epileptiform symptoms.

Diagnosis.

Finding of trypanosomes in blood (thick films), lymphatic glands (by puncture), or in cerebro-spinal fluid (centrifugalization) is conclusive evidence. There is evidence of cyclical appearance of trypanosomes in the blood and the disease may be missed if only one blood examination is made. Autoagglutination of red blood corpuscls, relative leucopoenia and increase of large mononuclears are present. Animal inoculation (rat or monkey), and blood culture on NNN medium, are helpful.

The disease should be differentiated from malaria, syphilis, kala-azar, and in later stages from cerebral tumour and encephalitis lethargica.

Prophylaxis.

Prophylaxis consists in isolation and treatment of the infected, control of people coming from infected areas, protection from glossina, removal of population from highly infected areas, destruction of tsetse fly and its breeding places, and destruction of animal reservoirs. Flies may be destroyed by trapping and catching, clearing of bush, etc.

Chemoprophylaxis by intravenous injections of Bayer 205 (antrypol) was considered to be effective, but later experience has shown that these do not always prevent infection. It was found in Congo, that intravenous injections of 1.0 gm in case of adults, and 0.3 to

0.7 gm in case of children, gave protection for some months. In other cases protection lasted for three months at least. Even if infection is not prevented the pathogenicity of trypanosomes is mitigated.

Prognosis. Untreated cases are invariably fatal. If adequate treatment is given in the early stages of the disease, the prognosis is good. It is now established that the disease is curable. Inadequate treatment with arsenical and antimonial drugs may give rise to resistant strains of trypanosomes.

Treatment. The earlier the disease is recognised and the treatment is started the better the chances of cure. The treatment with drugs should be regularly and adequately carried out and complications such as malnutrition, etc., should be promptly dealt with. The two effective drugs are Bayer 205 and Tryparsamide.

2. Chemotherapy of Trypnosomiasis

(1) Antrypol (Bayer 205, Germanin)

(1) *ANTRYPOL (Germanin, Bayer 205 NAPHURIDE)*

General. This is a complex organic combination (the symmetrical urea of sodium m-amino-benzol-m-amino-p-methyl benzol-i-napthylamino-4-6-8 trisulphonate. When used in veterinary medicine it is called *Naganol.* Fourneau prepared a substance of apparently identical action, named *Fourneau* 309, *Suramen* is also a similar preparation. This compound is believed to be identical with the German product. It is a derivative of the trypan-red class of dyes. *Antrypol* is a similar English product.

Germanin is a fine, white, flocculent powder which dissolves easily in physiological saline and cold water. The solution is faintly pink, odourless, slightly bitter in taste, and neutral to litmus. The drug can be sterilised by heating in a water bath for 15 minutes, but solution in sterile water renders this unnecessary. Solutions can keep for some length of time, but it is advisable to give freshly prepared solutions. The drug, both in a pure state and in solution, should be kept away from light in amber-coloured bottles. A 10 per cent solution is injected intravenously in man, and it is also said to be well borne subcutaneously, intramuscularly and orally. The intravenous route is preferable.

Pharmacology. Little is known about the pharmacology of this drug. It is slowly absorbed from the alimentary tract. In animals the drug has been found remarkably non-poisonous. The *dosis tolerata* has been estimated to be 160 times that of the *dosis therapeutica.* It could be given intrathecally to a dog in doses of 0.7 gm without producing irritation or other ill effects. The drug is said to circulate in the blood for some time without losing its efficacy. It probably combines with the proteins of the serum and in this way is fixed and retained. Its presence can be detected in the internal organs for as long as 5 months after treatment. As the drug circulates in the body for weeks or months, it is not necessary to repeat it too often. The trypanosomes develop a certain amount of resistance to it and it has toxic effects on the host. Its action on trypanosomes is said to be inhibitory to the multiplication of the parasites. The drug produces disappearance of the trypanosomes from the peripheral blood, and in this way transmission of infection to the flies is prevented, and the spread of disease is checked.

Mode of action. The mode of action of Bayer 205 is not properly understood. The drug produces no action on trypanosomes *in vitro.* After injection a trypanocidal substance can be demonstrated in the blood, milk, urine, etc., which may be the drug itself or a compound of it or some substance having no chemical relationship to it. A 1.0 per cent solution of the drug lessens or abolishes the coagulation of blood serum by heat. The drug has been considered by some to be directly parasiticidal. Kleiger and Weitzmann (1926) injected cultures of trypanosomes together with Bayer 205 in experimental animals and found that they manifested an increased resistance which in some of them sufficed to ward off an attack. This increased power of resistance is not attributed to the protective power of the drug alone as the same phenomenon was observed in infected animals treated with Bayer 205. It is suggested that the drug combines in some way with the trypanosome cell, producing a heterogenic antigen capable of stimulating the formation of specific antibodies. The action of the drug is not therefore purely parasiticidal. Along with this property there may be

an immunizing activity which completes the cure and affords an increased resistance in subsequent infections.

Toxicology.

Duncan and Manson-Bahr (1923) found that in mice, the drug in the kidneys produced extensive degeneration and exfoliation of the epithelium of the convoluted and other secreting tubules of the cortex. The straight tubules and excretory ducts appeared unaffected. Some of the tubules contained hyaline casts and there were necrotic foci. The blood vessels were engorged, minute hæmorrhages occurred in the cortex and vessels showed perivascular round-cell infiltration. The liver and the lungs were engorged and fatty changes and focal necrosis occurred in the former. Minute hæmorrhages also occurred in the lungs and in the brain. Marked anæmia was produced. In experiments in which the object is to produce sterilisation after a single dose, a great leucocytic reaction may be observed in relation to the sudden disappearance of parasites and death may result from shock. The drug circulates in the blood for days and even months, and consequently repeated doses have a cumulative effect. Regular examination of the urine is necessary and the appearance of a considerable amount of albumin is an indication for stopping the treatment.

Therapeutic use.

Germanin has had a thorough trial extending over many years, and its great value in early cases of the disease, when the central nervous system is still uninvolved, is firmly established. The utility of the drug varies with the type species of the trypanosome concerned and the stage of the disease at the commencement of treatment. In early gambiense infection, the drug is very effective but in its late stages a preliminary sterilization of peripheral blood by germanin has to be followed with tryparsamide. In the case of rhodesiense infections also, germanin is effective in early stages, but the treatment has to be continued with tryparsamide when the nervous system is involved.

It is generally given intravenously but it can be given intramuscularly. The intrathecal injection causes pain, vomiting, headache, convulsions and itchings even after small doses, and cannot be recommended. Besides, advanced cases are not benefitted by intrathecal injections.

The drug appears to be well tolerated and produces less toxic effects even in large doses in animals infected with trypanosomiasis than in normal animals.

The average dose by the intravenous route is 1.0 gm dissolved in 10 ccm of distilled water, the total amount necessary to effect a cure being 10.0 gm. The trypanosomes, however, disappear from the peripheral blood even after an injection of 0.5 gm, but reappear. This is the case with some of the other drugs and the doses have, therefore, to be repeated at weekly intervals.

Different workers have adopted different courses. Bayer 205 is given in doses of 1.0 gm on the 1st, 3rd, 10th and 13th days with good results. In severe cases individual doses of 1.5 to 2.0 gm have been given in adults. In Nigeria 1.0 gm doses of Bayer 205 followed by five 2.0 gm doses of tryparsamide was adapted.

Some individuals have an idiosyncracy to the drug, and collapse and death may result. It is, therefore, advisable to give a trial injection of 0.3 gm of Bayer 205 before a full dose is given. If an individual shows toxic effects, the drug is withheld.

Experience has shown that 0.102 gm of the drug per kilo body weight is usually well borne. The drug, however, has been shown to have a cumulative action and circulates in the blood for a long time, several months after a course of injections.

The drug is irritant to the kidneys and may produce nephritis; albuminuria

may occur after 3 to 4 injections, but this may be temporary and may pass off. In some cases the cerebro-spinal fluid improves several months after the treatment is stopped and the condition may remain stationary. In the majority of cases the disease progresses to a fatal termination. In those cases in which the cerebrospinal fluid is normal and the infection is confined to glands and blood stream, the drug is efficacious. The earlier the treatment is started the better.

Dosage and method of administration.

In human beings it is advisable to start with 0.5 gm intravenously and if it is well borne, to give 1.0 to 1.5 gm after 24 to 48 hours. The dose can be increased up to 3 gm but it is not advisable to give such large doses more than once a week. One gramme is usually given weekly, in most cases repeated two or three times. Larger doses should only be given when the drug is well borne. In children proportionately smaller doses, *viz.*, 0.4 to 0.6 gm are given. The drug has been given by the oral route and trypanosomes may disappear from the blood but they reappear. If trypanosomes reappear after the 4th or 5th injection it is better to change to another drug. The drug cannot be given intrathecally, as it is likely to produce alarming symptoms. According to some weekly intervals between injections allow the trypanosomes to become resistant.

The trypanosomes do not disappear from the blood so rapidly as under tartar emetic, atoxyl, etc. As a rule, the blood is not free from the parasites till the second or third day after the injection. Frequent examinations of thick blood films or centrifugalisation of blood in citrated saline are necessary in cases of relapse to demonstrate the presence of the parasites. Sometimes isolated parasites appear after a few weeks but these disappear without further treatment. The best test for cure is to inject the infected blood into small experimental animals and see if the disease can be transmitted. Clinically, the action of the drug is apparent by disappearance of the glandular swellings in the neck, diminution in the size of the spleen, improvement of the blood picture, increase of body weight and general mental well-being.

Toxic effects.

Although toxic reactions are remarkably few in number, the drug should be carefully administered. Vomiting may occur if it is injected too rapidly. Renal epithelium may be damaged and this is by far the commonest toxic effect of the drug. Albumin in the urine generally occurs after the third or 4th injection, there may be blood and casts, and fatal anuria may occur. Symptoms of urinary irritation may subside after the termination of the course of treatment, but occasionally they persist. When patients are undergoing treatment, a careful watch should be kept on the blood and the urine. The presence of albumin in the urine gives a warning, although many authorities do not consider it a contra-indication to the use of the drug; care, however, is indicated in such cases. Albuminuria may sometimes occur even after small doses. In many cases it disappears after a few doses without leaving any permanent after-effects. Some authorities do not hold albuminuria to be necessarily an indication for the interruption of the treatment in patients whose kidneys are at other times normal. An erythematous rash sometimes appears, commencing on the forearms and spreading to the rest of the body. The eruption consists of central raised papules, rather patchy in distribution, and finally it disappears by desquamation. Pruritus is a constant accompaniment, and purulent conjunctivitis and stomatitis may occur. Cases of sudden collapse have been recorded. In some cases hæmolysis is produced. Optic troubles have not infrequently been observed. The Sleeping Sickness Commission in Portuguese West Africa in 1923 concluded from their observations, that though Bayer 205 caused rapid disappearance of the trypanosomes and nephritis in some cases; amblyopia leading to complete amaurosis, militated against its general employment,

rophylactic es.

Experiments conducted in Africa show that Bayer 205 and Fourneau 309 have a definite prophylactic value. A dose of 2 gm of germanin given to an adult is said to protect against any of the two trypanosomes for a minimum period of three months. Within certain limits, administration of two or three small doses at intervals of a few weeks each, offers better protection than the administration of one big single dose. Protection against rhodesiense infection is relatively greater than gambiense infection. Other workers consider that protection is not of more than six months duration. Administration of the drug in doses of 1.0 gm every two or three months is recommended in those entering highly endemic areas.

The efficacy of chemoprophylaxis may be due to the fact that though rapidly excreted or destroyed, the chemical compound may lead to a long persisting elevation of the defensive powers of the tissues. In the case of trypanosomiasis there is no evidence in support of this mode of action. The compound may be excreted or destroyed so slowly that it continues to circulate and exert a trypanocidal activity for a long period, or finally it may form a relatively insoluble local deposit in the tissues from which small amounts continually pass into the body fluids to act on the parasites.

Comparative prophylatic value.

In order to be of value as a chemoprophylactic against sleeping sickness for any length of time, a drug, it appears, must maintain a trypanocidal titre in the serum. Since the trivalent arsenicals and arsphenamines produce a high trypanocidal titre in the serum for only a comparatively short period, they should, on theoretical grounds, be of little value in prophylaxis, and this, in fact, has been shown to be true experimentally. Quinquevalent arsenicals, on the other hand, are present in the serum in a trypanocidal titre for a much longer period; tryparsamide thus exerts an efficient prophylactic action against nagana infections when given by mouth to rabbits. The effect of tryparsamide in preventing sleeping sickness in man has been tested, and 2 gm of tryparsamide were given intravenously. The period of protection lasted for approximately six months and the number of fresh infections among those prophylactically treated was twelve times less than among the untreated controls. Even so, the results were not as successful as with germanin. The prophylactic action of germanin is due to the fact that concentrations in the blood of 10 mgm per 100 ccm and not infrequently as low as 1 gm per 100 ccm, are sufficient to inhibit the power of trypanosomes to infect the mouse. It has been shown that concentrations of 1 mgm of germanin per 100 ccm can usually be detected in the blood five to six months after intravenous injection of a rabbit with 100 mgm per kilogram of body weight. The amount of germanin in the blood is usually greater when a number of small doses of germanin are given over a period of a few weeks than when the same amount of the drug is given in a single injection. (Findley Chemotherapy, 1939).

(2) Other Drugs in Trypanosomiasis

(a) Diamidino Stilbene

This compound has been tried in Trypanosome infections. Of 13 sleeping sickness cases treated at Gandau during 1939 with diamidino stilbene, three mild or moderately severe cases were clinically cured and one improved, though in no case did the cerebrospinal fluid, when initially pathological, return to normal. Of the remaining nine moderate or severe cases, one was clinically unchanged and eight were definitely worse. The cases seen at Gandau are, in general, of a severe type and require a long course of antrypol and tryparsamide before apparent cure is obtained; even then the C. S. F. is usually just outside normal limits. Diamidino stilbene is, therefore, subject to a very severe test when

tried out on such cases. The results are not very encouraging and do not justify the hope that the drug will prove as efficacious as antrypol and tryparsamide, even if used in large doses over a long period.

(b) Antimony Compounds

Antimonyl tartrates have a remarkable effect in diseases produced by trypanosomes in animals. It was found that 1 mgm of antimony caused immediate disappearance of the parasites in rats weighing 200 gm. Plimmer and Thomson (1908) noticed the sterilising effects of potassium and sodium antimonyl tartrates in laboratory animals infected with *T. brucei,* and *T. evansi.* Rats inoculated with nagana died in an average time of 5 to 6 days. If on the fourth day, when the blood was full of parasites, 5 mgm of tartar emetic were injected subcutaneously, the parasites, were killed and the rats usually survived, and some recovered completely; relapses, however, not infrequently occurred. Arsenic and some aniline dyes produce the same type of effect, but are inferior to antimony compounds in this respect. A large number of other antimony compounds were also tried in the treatment of this infection in animals, and rapid disappearance of the parasites after intravenous injections, was observed. The effects were so rapid, that there appears to be little doubt that the trypanocidal action is due to antimony, and not to the formation of antibodies. No evidence of agglutination or phagocytosis has been found.

The immediate trypanocidal action of the trivalent antimony preparations *in vivo* (also highly effective *in vitro*) as compared with the delayed action of the pentavalent preparations, revealed an essential difference in the action of these two groups. In the same way as with arsenical compounds, a reduction of the pentavalent preparations to the trivalent form is assumed to take place within the system.

Antimony compounds were first tried in the treatment of human trypanosomiasis many years ago. Borden (1910) treated sleeping sickness in the Congo with injections of tartar emetic. Kerandel treated himself with injections of tartar emetic after failing with atoxyl. Owing to the chronicity of the disease, and the great tendency to relapse after long intervals of absence of the parasites from the peripheral circulation, it is very difficult to draw any conclusion regarding the curative effects of the drug. The successful treatment of trypanosomiasis entails strict control over the patient, possibly for two years. The treatment of sleeping sickness with antimony has receded into the background as compared with Bayer 205 (Germanin).

In the treatment of animal trypanosomiasis antimony is also used in the form of antimosan veterinary. In Nagana, an important trypanosomal disease of cattle, antimosan is effective both as a curative and prophylactic drug. It may be used alone or in combination with Bayer 205 (Germanin).

(c) Arsenicals

Arsenicals.

Tryparsamide is a valuable drug in the treatment of advanced cases. Orsanine is a French equivalent. The drug is a colourless crystalline solid, freely soluble in water forming neutral solution. It may be given intramuscularly or intravenously, the latter method being preferred. The average dose is 0.045 gm to 0.05 gm per kilo body weight and even with this dose ocular symptoms may develop. It is recommended by some authorities that the starting dose should be 0.04 gm per kilo body weight. Children stand the drug well, almost an

adult dose being tolerated. The drug, to be effective, should be pushed to limit of safety, and 15 weekly injections of 0.045 gm per kilo is the usual course though some only give 12 injections.

A second course should be given after 1 to 3 months have elapsed after the first course.

Tryparsamide is less effective if previous injections of arsenicals, such as atoxyl, have been given.

Tryparsamide is an efficient trypanocide. Chesterman (1923) treated 40 cases in the Belgian Congo by 8 to 10 weekly intravenous injections of 3 gm each dissolved in 10 ccm of water, with good results. The dose should not exceed 4 gm, and intrathecal administration is not recommended. Adults receiving less than 0.05 gm per kilo body weight generally relapse; children can stand comparatively much bigger doses, and in these cases 0.1 gm, per kilo, can be given.

Tryparsamide gives comparatively much better results than Bayer 205 in the treatment of trypanosomiasis when the central nervous system is affected. It clears the blood in from 6 to 12 hours in doses of 1.5 gm, intravenously, in early infections due to *T. gambiense* and the peripheral blood remains free for long periods sometime. The drug is not effective in infections with *T. rhodesiense*.

Weekly injections of 2 to 3 gm in adults have been recommended because such doses give equally good results as large and more frequent doses, and because they reduce the chances of ocular disturbance to the minimum. In the first stage of the disease a total dosage of 20 to 50 gm suffices to produce a cure. The blood and glands become negative; there is marked clinical improvement in those cases in which the spinal fluid is only slightly changed before treatment, and it tends to become normal with the treatment. In the second stage it is necessary to give 50 to 100 gm. The patients who exhibit nervous and mental symptoms are said to show rapid and considerable improvement; in cases of moderate intensity the benefit obtained is marked and constant. The action is said to be rapid, durable, constant and is superior to other drugs. Relapses or incomplete cures are always due to extraneous causes such as insufficient dosage, irregularity of the injections and difficulties due to the patient. Toxic reactions, acute or chronic are negligible. Blindness which occurs after treatment with tryparsamide is in most cases due to previous arsenical treatment. Blindness does not usually come on suddenly and if patients are watched, it is mostly transitory. The premonitary symptoms are pain in eyeballs, photophobia, lacrymation and dimness of vision. If these occur, the drug should be stopped, or the interval between injections lengthened. The danger of blindness is increased as the cases are advanced and nearing death.

Tryparsamide is said to have a beneficial effect on general nutrition in addition to its parasitotropic effects, and some clinicians believe that its chief action is to build up the resistance of the body to combat infection.

Prophylactic use.

Tryparsamide has not been extensively used as a prophylactic drug in sleeping sickness in man. In vaginal infections in animals (rabbits) it has a prophylactic effect.

Arsenicals.—Trypanosome infections were first treated with preparations of arsenic by Bruce (1895) and Lingard (1899) who employed them in animal trypanosomiasis. Later, Laveran and Mensil (1902) used in organic arsenic in an attempt to cure trypanosomiasis in laboratory animals.

Atoxyl (soamin).—Atoxyl was employed by Koch (1907) in Africa in the treatment of sleeping sickness. The preliminary reports inspired great hopes, but later it was found that relapses frequently occurred, while optic atrophy was by no means uncommon. For details see the chapter on arsenic.

Bismuth in trypanosomiasis.—Colloidal bismuth and a basic oxy-aminophenyl arsinate of bismuth have been tried but the results are not encouraging.

Quinoline derivatives.—Anil and steyryl-quinoline derivatives and surfen C have been tried in trypanosomiasis. These compounds are of some use in animal trypanosomiasis only. Clinical results have not been satisfactory.

Acridine derivatives.—Acriflavine has long been known to possess trypanocidal properties.

The action of a number of pyrrole dyes has been tested (1935) on *T. brucei* infections in mice. Pyrrole blue, pyrrol red and phthalocyanin have no trypanocidal action but the perchlorate of a red dye obtained from Bacillus prodigiosus and termed prodigiosin was found to have a temporary curative action.

Phenanthridinium compounds.—Some phenanthridine and phenanthridinium compounds possess trypanocidal action and act on a strain of *T. brucei* completely resistant to the largest tolerated doses of arsacetin.

These compounds have been tried in trypanosomiasis.

Bayer 9736 contains 22 per cent of arsenic and 5 per cent of sulphur. The dose is 1.5 ccm of a 10 per cent solution to adult increased to 3.0 ccm. It is given intravenously twice or thrice weekly till 50 ccm are given. It is effective against T. gambiense and T. rhodesiense.

3. Summary

Trypanosomiasis in man is a curable disease if it is early recognized and adequately treated. Bayer 205 and tryparsamide are the two most effective drugs.

Bayer 205 is given intravenously in average doses of 1.0 gm till 5 to 10 gm are given, the course lasting a month. It is advisable to give a trial dose of 0.3 gm to test the sensitiveness of the patient especially in advanced cases; injections are then given on the first, third, tenth and thirteenth days, after which they are given at weekly intervals till the total amount is given.

Tryparsamide is given in doses 0.5 to 1.0 gm by the intravenous route at weekly intervals till 30 to 50 gm are given, the course lasting 1 to 3 months. In advanced cases it is advisable to give a small trial dose to test the sensitiveness of the patient to the drug. The drug should then be rapidly pushed to its tolerence. In cases where the central nervous system is affected a second course must be given after 1 to 3 months rest.

Dosage for children up to 12 years is comparitively larger than for adults.

Combined treatment with Bayer 205 and Tryparsamide is indicated in cases where the former fails.

Evidence of cure are, general improvement of the condition, reduction of temperature, normal cell count and albumen content of the cerebro-spinal fluid. After treatment the patient should be examined every three to six months for 2 to 3 years.

The antimony compounds have their chief application in veterinary practice.

4. South American Trypanosomiasis

(Also known as Chaga's disease occurs in Panama, Mexico, Brazil, Argentine and other parts of South America).

The causal organism is the small C shaped *T. cruzi* occurring sparsely in blood. The disease specially affects children and acute and chronic forms have been described. The parasite develops in the endothelial cells of the body tissues especially heart, internal organs and striated muscle, and destroys them. There is rapid multiplication and formation of nests of leishmania which later develop into trypanosomes. These invade the blood stream and occur as broad and slender forms.

Transmission. The parasite develops in the reduviid bug especially *Triatoma megista* now classified as *Panstronglus megistus,* and also in *T. infestans* in North Argentine. The trypanosomes ingested pass through the gut, and in 8 to 10 days fully develop metacyclic forms appear in the hind gut and pass out in the fæces. Infection occurs when the bugs, which are vicious biters, bite and defaecate in the wound. Transmission in the bug is hereditary and parasites develop in larval and nymphal stage. The domestic cat is infected, and under experimental conditions all laboratory animals can be infected. An animal named 'tatu' Tatusia or *Dasypus novemcincta,* whose body, legs and tail have an armour, is said to be the host; five species of this animal are said to be naturally infected with the parasites. Transmission is also said to occur through milk of infected mothers and through coitus.

Pathology. The heart is affected and enlarged; pericardial effusion and myocarditis are present; the liver shows parenchymatous degeneration; the brain and cord show sub-serous echymosis and haemorrhages; adrenals are also involved. In acute stages there is high leucocytosis with increased mononuclears and severe anæmia.

Clinical aspect. The severity of the disease varies in different localities. In many cases in which the organism is found in the blood, there may be no evidence of disease or of recent illness, though mild febrile disturbances may have occurred. At the site of inoculation, swellings Chagoma form and subsequently metastatic tumours occur. Unilateral opthalmia with palpabral œdema and conjunctivitis, *'Romanos sign,* is probably due to the entrance of parasites through the conjunctiva. The incubation period is between 7 to 14 days possibly longer. In experimentally infected human beings it was found to be 10 to 12 days.

The acute type of disease is seen in children in the first year or two of their life. There is fever rising to 104°F (40°C), œdema of face, and unilateral conjunctivitis due to the bite of the infected bug. The œdema may spread from the face to the rest of the body. When flagellates disappear from the blood, the temperature becomes normal. There is general adenitis, and the spleen and liver are enlarged; death occurs from myocarditis produced by invasion of the heart-muscle by the parasite. Encephalo-meningitis is a terminal event.

The chronic form is produced if the child recovers from the acute stage. There is enlargement of lymphatic gland and thyroid (this may be endemic goitre), loss of hair, dulness, myxoedema, apathy, alteration in speech and other nervous disorders. If the heart is involved, cardiac irregularity may be striking, and even heart block may appear. Some consider that cardiac disturbances may be due to vitamin deficiencies.

Diagnosis. Diagnosis depends on finding C shaped trypanosomes in the blood, especially during the febrile stage. When parasites are not found, inoculation of 5 to 10 ccm of blood into susceptible animals, (guinea pigs, puppies) or culture in NNN medium is advisable. Examination of muscles, liver heart with a viscerotome may be done.

Complement fixation test of Machado with antigen made from glycerine and water extract of heart and spleen of an infected animal, is said to be specific. Brumpts xenodiagnosis test consists of an infection—free Triatoma biting a patient; its gut is examined after two weeks for the parasites. Differential diagnosis from kala-azar is important.

Prophylaxis. Protection from bites of the bug by means of mosquitoe nets is the best prophylactic measure. Bugs hide themselves in crevices, roofs, etc., of the huts and are difficult to get at. If possible, destroy infected huts. The bug is never found away from human habitation.

Many deaths occur in the acute stage in young children. In adults, cure may occur after the acute stage, but Chagas believes that the disease passes on to chronic stage. In older children and adults, infection produces mild symptoms. Outlook in cases associated with endemic goitre is complicated. *Prognosis.*

Treatment is unsatisfactory with all drugs found favourable in African trypanosomiasis. A number of new chemotherapeutic drugs of the amino-quinoline series, *e.g.*, surfen C, are very toxic and useless. Bayer 205 (antrypol), undecaine-I : II-diamidine, arsenicals and antimonials, atebrine are ineffective. Bayer 7602, a quinoline derivative has been tried in Brazil and is said to have definite therapeutic activity. The drug is given intramuscularly on alternate days, as a freshly prepared 3 per cent solution, in doses of 5 ccm for adults rising to 20 ccm, a total of 22.2 mgm per kilo body weight is sufficient to effect a cure. Five injections are given on alternate days. The drug is said to reduce leishmanial figures indirectly by direct action on trypanosomiasis. *Treatment.*

Bayer 9736 is another drug which contains 22 per cent of arsenic and 5 per cent of sulphur. It is less toxic than 7602 and is given intravenously in 10 per cent solution. The adult dose is 1.5 ccm (0.15 gm) increasing to 3.0 or even 4.5 ccm, given twice or thrice weekly to a total of 50 ccm (5.0 gm) for men, 40 ccm for women and 30 ccm for children.

M3024, I. C. I. is the British equivalent of Bayer 7602. Initial dose 7-12 mgm per kilo usually 0.15 gm; 2 to 4 ccm given at 5-7 days intervals, the second dose being double of first. Improvement is said to be rapid. Penicillin used locally and intramuscularly and a total 4 to 5 hundred thousand units gives good results.

Rats recovered from the disease are immune to re-infection. Administration of serum of recovered animals does not prevent infection but reduces the severity of symptoms and reduces the number of trypanosomes in the blood.

When the disease is complicated with myxœdema, treatment for the latter condition should be given.

CHAPTER III

LEISHMANIASIS

Kala-Azar

CLINICAL ASPECTS—DIAGNOSIS—SPECIFIC TREATMENT: ANTIMONY DERIVATIVES, ANTIMONY COMPOUNDS, ACTION OF ORGANIC ANTIMONY COMPOUNDS—ANTIMONY IN VISCERAL LEISHMANIASIS: PENTAVALENT COMPOUNDS, ANTIMONY RESISTENCE, EVIDENCE OF CURE—MODE OF ACTION—ROUTES OF ADMINISTRATION—TOXIC EFFECTS—SUMMARY—AROMATIC DIAMIDINES—TREATMENT OF RESISTANT CASES—TREATMENT OF COMPLICATIONS—TREATMENT OF POST-KALA-AZAR DERMAL LEISHMANIASIS.

Cutaneous Leishmaniasis

ORIENTAL SORE—MUCO-CUTANEOUS (SOUTH AMERICAN LEISHMANIASIS)—LEISHMANIAL DYSENTRY.

General Consideration

General.

The term *Leishmaniasis* includes a number of conditions occurring as a result of infection with various species or varieties of species of genus Leishmania namely:—

1. *Visceral Leishmaniasis*

   (*a*) *Indian Kala-azar* caused by *L. donovani* which spreads by the blood stream into all the body tissues except the nervous system.

   (*b*) *Infantile kala-azar* caused by *L. infantum* which has now been shown to be the same as *L. donovani*. *L. chagasi* which produces South American Kala-azar is also indistinguishable from *L. donovani*.

2. *Cutaneous Leishmaniasis*

   Oriental Sore. The causal organism is *L. tropica* which usually does not spread by blood stream.

3. *Muco-cutaneous Leishmaniasis*

   Espundia. The causal organism is *L. brazilensis* which spreads from a primary invasion of the skin after it has healed to nasal, buccal and pharyngeal mucous membranes.

4. In addition, dermal leishmaniasis may occur as complication or as a sequel to Visceral Leishmaniasis particularly as a post-kala-azar condition after treatment with antimony compounds.

According to some workers there is no hard and fast line of demarcation between these types. Thus in Kala-azar, there is a primary skin lesion which instead of leading to permanent healing and solid immunity, as in case of oriental sore, leads to the generalized disease Kala-azar. Similarly in muco-cutaneous leishmaniasis, the primary lesion occurs on the skin which may behave like oriental sore in some cases, while in others secondary lesions in mucosa of the nose, mouth and pharynx may develop. Transition forms in between the three clearly defined types of leishmaniasis are seen.

This section is largely based on the classical work of Dr. L. E. Napier on leishmaniasis in India as summarised in his "Principles and Practice of Tropical Medicine," 1943.

KALA-AZAR

Kala-azar is an infective disease characterised by persistent fever leading to enlargement of spleen and liver and cachexia. The disease is widespread in many parts of Asia. In Europe it occurs in countries bordering on the Mediterranean; it is prevalent in parts of Africa. It was a terrible scourge in India, being confined to low lying alluvial plains. Assam, Bengal, Bihar and Madras are still heavily infected countries. The limits of endemic areas are well defined being confined to the eastern side of the Peninsula. The disease is endemic in China from Peking in the north in the region of Yangtse-Kiang River, to as far south as Canton; cases have been reported from Mongolia.

Epidemiology.

Kala-azar is endemic in Bengal and Assam; here it was subject to exacerbations of epidemic-like nature which had a definite periodicity. On account of extensive treatment campaigns, the character and periodicity of these epidemic waves is being disturbed both in Assam and Bengal. The disease may show true epidemic invasion in an area and three of these epidemics have been recorded in Assam during the last fifty years.

Kala-azar is usually confined to areas below an altitude of 2,000 feet and prefers alluvial soil and a mean annual humidity of not less than 70 per cent, with a low mean diurnal range of temperature of not less than 20°F. It is a disease of rural areas rather than towns. It is a family, house and site infection.

There is definite seasonal incidence and in Bengal and Assam there is a peak in the winter months, but it starts to rise in the autumn during the period immediately following the rainy season.

The disease in the Mediterranean occurs in 93 per cent of cases in children under 5 years of age and is rare among adults. In Asiatic endemic areas, the highest incidence is between the ages of 5 and 15 years. Even in India cases of Kala-azar occur among infants. The disease is rare among better class Europeans and Indians, but common among poor class Europeans and Anglo-Indians.

Aetiology.

The disease is produced by a protozoon of the family Trypansomidae and the genus *Leishmania—L. donovani.* Two forms of parasites are known, the non-flagellate and round, the Leishman-Donovan body which occurs in the mammalian host, and the flagellate form occuring in its arthropod host. In culture medium round forms develop into flagellate forms. The L. D. body is found in the endothelial cells and large wandering macrophages in all parts of the body. The parasite is found in the spleen, liver, bone marrow, lymphatic glands and submucosa of the intestinal and respiratory tracts at sites which are rich in reticulo-endothelial cells. In the blood, they occur in polymorphonuclear leucocytes where they are being phagocytosed, and in the large mononuclears, where they are multiplying (Napier). The leishmania multiply by biniary fission in the cells of the host, and as many as 200 parasites may occur in a single cell. Parasites have also been found in the urine and fæces.

The most satisfactory medium for cultivation is the NNN which is a saline agar preparation to which one-third of fresh rabbits blood is added. The growth occurs in the water of condensation after incubation at 20-40°C; in cultures they assume a flagellate form called *leptomonas.* In susceptible animals, *e.g.,* Chinese and Syrian hamsters, infection can be produced by subcutaneous, peritoneal or oral routes.

Transmission.

The sandfly *Phlebotomus argentipes, P. major var. chinensis* and *P. perinciosus* are credited with the transmission of this disease. The parasite is taken into the mid-gut with the blood meal, and here the round forms become flagellate and active division occurs; by the 4th day the mid-gut becomes blocked with a plug of the flagellates, on the 5th day they reach the pharynx and on the 7th day into the buccal cavity. During the course of feeding, the sandfly injects the parasites into the wound made by the probocis. The flagellates thus get into the blood, are taken up by reticulo-endothelial cells, enter the circulation and are carried to the spleen, liver, etc.. and general infection occurs.

The sandflies are found in the ground floor rooms of houses with unpaved floors, which are damp and badly ventilated and in cattle sheds. They breed in any earth that contains admixture of nitrogenous matter. They prefer to feed on cattle and bite man when these are not available. In the laboratory the sandfly breeds at a constant temperature of 80° to 82°F.

It is believed that in India man is the main source of infection and the patients treated and cured of the disease may still be source of infection. In the Mediterranean area and

China infected dogs may act as carriers. In India canine infection occurs in Northern India where Kala-azar does not occur (Napier).

Immunity.

Natural immunity in Kala-azar does exist; that there is acquired immunity is evident from the fact spontaneous cures occur in this disease. There also appears to be little doubt that there is gradual development of immunity established by previous infection recorded in dogs after cure. It has been shown that there are differences in the antigenic structure of *L. donovani, L. tropica* and *L. brazilensis,* but *L. infantum* was antigentically identical with *L. donovani.* No agglutinins are found in the serum of the patient, but there is evidence of some natural immunity to infection. When the disease is cured, the patient enjoys protection from a second attack. There is no cross immunity against other leishmanial infections, *e.g., L. tropica.* (Napier).

General lowering of resistence is an important factor in contracting the disease and debilitated individual are usually picked out. In epidemics of Kala-azar in India, circumstances, which have periodically lowered the general vitality of the people, have been determining factor.

Pathology.

The parasites occur in all parts of the body in areas rich in reticulo-endothelial cells. Macrophage proliferation actually preceeds parasites and all changes observed in different organs are due to proliferation of reticulo-endothelial tissues, the fibrotic changes occuring in organs, such as the liver and spleen appear late and are not constant (Napier). The spleen is generally enormously enlarged and may weigh 8 to 10 lbs or more. There may be perisplenitis and thickening of the capsule; its consistency is not hard (like malaria) and it is friable; the cut surface is uniformly dark-red; there may be infarcts.

Histologically there is infiltration with heavily parasitized macrophages which dominate the picture; they encroach into the Malpighian corpuscles which completely disappear; the vascular spaces are enlarged.

The liver is enlarged, firm, friable and has a greasy look as in fatty degeneration; Kupfer's stellate cells are enlarged and parasitized and proliferated, especially in the portal zone and portal spaces. In the central zone the capillaries are dilated and both the liver cells and the reticulo-endothelial cells are atrophied and fatty changes are present in them.

In the intestines, proliferation and parasitization of reticulo-endothelial cells may occur in the submucosa.

In post-Kala-azar dermal leishmaniasis, the subpapillary layer is œdematous and the vessels are enlarged, dilated and infiltrated with macrophages. Parasites do not occur in early lesions in large numbers; they occur in the cutaneous nodules.

The blood shows a red cell count of about 2 to 3 millions, reticulocytes are above normal (2 to 4 per cent), and the fragility of red cells is decreased to hypo-and hypertonic saline solutions. The leucocyte count is usually below 4,000 per ccm, normal ratio of 1: 750 red cells being reduced to 1: 1,000 to 1: 1,500. Agranulocytosis is rarely seen; eosinophils are usually 1 to 2 per cent; there may be an absolute increase of large mononuclears. There is decrease in blood platelets to 200,000 per ccm; the coagulation time is reduced from 5 minutes to about 3. The sedimentation rate (ESR) is increased, the mean reading was 68.3+11.2 mm. The pH of the blood is above normal and the alkaline reserve is reduced and so is the blood calcium; the blood sugar may be as low as 0.05 per cent; there is an inversion in the albumin-globulin ratio, which reverts to normal after treatment. There is nearly always a trace of albumin and marked increase in urobilin in the urine (Napier).

1. Clinical Aspects

The incubation period is usually between six weeks to four months, but cases have been recorded in which it has been as short as 10 to 14 days or as long as 18 months. A case of congenital infection has been reported.

The onset may be acute or insidious. In India it may be enteric-like or malaria-like. There is general malaise and the temperature rises rapidly up to 103 to 104°F in a week or so; this is maintained for a week or 10 days and then gradually falls to 99°F or even to normal. The temperature assumes the form of long irregular waves; it may remain low for a week or so, and then gradually goes up again. The double diurnal rise is observed in less than 10 per cent of the cases; in some there is a third diurnal rise; to demonstrate this, temperature has to be taken every three hours. In the second febrile attack the temperature may be remittent or intermittent and the classical double rise may appear. The spleen is palpable; pulse is 120 per minute and the patient is not toxic.

In the malaria-type, the onset is more acute and fever may be accompanied by a rigor; this fever may even respond to quinine during the first attack, but not so much in the second attack and afterwards.

In the insidious type, the time of onset cannot be determined but the patient gets attacks of irregular fever and comes to the hospital with an enlarged spleen with some such complications as dysentery or pneumonia; the condition is discovered accidentally. The serum test shows that the disease is in an advanced stage.

Cases are met with transient infection, the parasites being demonstrated by blood culture, but symptoms subside even if no treatment is given; these patients may not return for 3 or 4 years with any symptoms of disease (Napier).

Signs and Symptoms.

When the disease is established and has progressed for six months or so, there is fever with progressive loss of weight, weakness, increasing pigmentation in the skin, falling of hair, shortness of breath, palpitation, attacks of diarrhœa, bleeding from the gums and irritating cough. The patient may look well nourished or emaciated, and there is dark pigmentation of the skin in the region of the forehead and around the mouth; the hair is dry, lusturless and sparse and may entirely disappear. There is visible pulsation of the carotids in the neck; the abdomen is prominent on account of the splenic enlargement which is a most constant feature. The peculiar soft doughy consistency of the spleen is characteristic of Kala-azar except in rare cases when it may be hard. There is usually some enlargement of the liver. Jaundice is not common in the early stages of the disease but may be present later (Napier).

The typical fever is present but apyrexial cases may occur. Even with a temperature of 102°F or more, symptoms are slight and the patient may be doing his work in the ordinary way. Headache may be present but is usually not severe; no mental dullness is present. Retinal haemorrhage may occur.

The blood pressure is usually low, about 100 mm systolic; pulse is 100 to 120 per minute. The heart shows some dilatation or may even hypertrophy; haemic murmers are frequently present; oedema of extremeties is common. Ascites may occur in a small number and is a bad sign from the point of view of prognosis. Bleeding from the gums and epistaxis commonly occur; appearance of purpuric spots is uncommon and is a terminal symptom; it may be accompanied by uncontrollable hæmorrhage from the gums and the intestines; retinal haemorrhages rarely occur.

Gingivitis with loosening of teeth is common and cancrum oris is not uncommon during the late stages in fatal cases.

Diarrhœa and dysentery are common complications and a specific leishmanial dysentery has been described; a terminal dysentery may occur. In later stages congestion of lungs and broncho-pneumonia may occur. The nervous system is not affected in this disease and the patient is mentally quite clear even in the final stages. Herpes zoster may sometime occur.

There is no renal insufficiency and such symptoms as swelling of the legs and puffiness of face may be due to vasomotor disturbances. In women, amenorrhoea is an early symptom; an uncomplicated pregnancy may go on to full term (Napier).

Sequelae.

After proper treatment most cases recover completely, post-kala-azar dermal leishmaniasis being the only sequel of importance. This condition occurs in about 6 per cent of cases in old endemic areas of Kala-azar after full treatment with antimony compounds. It is believed that in a number of cases leishmania persists in the skin after cure of the visceral infection. This is of epidemiological importance. Usually about a year, but sometime several years elapse before the dermal infection is manifested.

Post-Kala-azar Dermal leishmaniasis.

It has no relation to the oriental sore. Hypopigmented macules may occur on the trunk, arms, thighs, legs, abdomen, which may resemble leucoderma, but the depigmentation is never so complete. The butterfly erythema may occur on the face, nose, cheeks and chin, the area involved being photosensitive. Nodules may occur on the face, lips, forehead, etc., and may be mistaken for leprosy; they rarely ulcerate.

Complications: Lowered body resistance may produce bacterial invasion resulting in cancrum oris, septicæmia, pneumonia, cystitis, otitis, etc., anæmia and agranulocytosis may

occur. Diarrhœa and dysenteric symptoms may be produced by influx of the parasites into the intestinal mucosa and secondary infection of the ulcers is thus produced.

2. Diagnosis

In endemic areas, diagnosis can be made from the clinical picture but it can only be established on demonstration of the parasite. Splenomegaly, irregular pyrexia, bleeding from nose and gums, anorexia, loss of strength, emaciation, pigment spots, cancrum oris, etc. give indication of the disease. Leucopenia is an important positive therapeutic test, *i.e.*, response to a few injections of antimony is not dependable and, therefore, not justified because it takes a long time before the cases show improvement under antimony treatment. However, quinine proving ineffective in reducing fever, may prove useful. In view of the simplicity of serum tests it is always advisable to confirm the diagnosis made on clinical grounds. The serum tests are a sufficient evidence for all practical purposes when the disease is developed, but are not so helpful in early cases. The demonstration of *L. donovani* in the blood, liver, spleen or bone marrow is the only final proof of the disease and is necessary in early cases of kala-azar.

arasites in the eripheral blood ms.

Repeated examination of blood films, made with special care, to get a straight leucocytic edge and stained by Leishman or Giemsas stains will help in establishing diagnosis in 60 to 70 per cent cases. An intravenous injection of an organic antimony compound increases the chances of finding L.D. bodies provided the film is prepared within half an hour of the injection (Chopra and Dasgupta).

lood Culture.

Under very strict aseptic precautions blood is taken from a vein and about 0.5 ccm is added to 10 ccm of saline containing 2 per cent sodium citrate. The cellular deposit after setting is then sown into NNN medium (rabbits-blood agar slope with condensation fluid); the tubes are incubated at 20 to 24°C for at least a week, and drops of the condensation fluid are examined at intervals in a fresh state; in positive cases, flagellates are seen moving about amongst the red corpuscles. Cultures, however, can not be discarded before a month. If proper care is taken the results are successful in almost all cases (Napier).

pleen puncture

Spleen puncture is the most important method of diagnosis and if performed with reasonable care in properly selected cases, is not at all a dangerous procedure, particularly in experienced hands.

Calcium lactate 30 gr is given the previous night in two ounces of water, and again next morning; no food is allowed. The puncture is done with a 5 ccm syringe and No. 11 needle about 1 to 1½ inch in length. The patient lies flat on his bed and the operator sits on the edge of bed on the left of the patient. A point is selected half an inch below the costal margin about the centre of the parietal surface of the spleen. After carefully sterilizing the skin at the site of puncture with pure phenol which is wiped off with a spirit swab; a puncture is made in two movements, with the first movement the skin is punctured and with the second the needle is plunged into the organ. After puncturing the skin, the direction of the angle of the needle is changed to an upward and outward direction paralell with the long axis of the spleen and at an angle of 45° with the skin surface. The needle is then plunged into the spleen and the piston is withdrawn rapidly two or three times, the needle is then withdrawn altogether. The contents of the needle are put in a slide and into the culture medium. A special attachment, which can be held and worked single handed, has been devised. After the puncture, a binder is applied and calcium lactate 30 gr is given; the patient rests in bed for the day and is allowed food one hour after the puncture. Spleen puncture according to Napier is the sheet anchor of diagnosis in Kala-azar and gives 95 per cent positive results by smear and 100 per cent by culture (Napier).

ternal puncture.

Liver puncture, according to some, is less dangerous but parasites are difficult to find; it is now discarded in favour of sternal puncture. The best site for sternal puncture is just on one side of the middle line at the level of the second intercostal space. A Salah needle of rustless steel is preferable and an 18-gauge lumber puncture needle may be used. Usually a guard has to be fixed at a distance of 1 to 1.5 cm. The skin is first cleaned and the subcutaneous tissue is infiltrated with a 2 per cent solution of novocaine or percaine; the bone is then pierced with a rotary movement; often considerable force is necessary, when the marrow cavity is reached there is loss of resistance. The stylet is then taken out and with a 2 ccm record glass syringe aspiration is made. When the fluid is aspirated the patient has a dragging pain which shows that the needle is in the marrow cavity. Only a few drops of the blood are removed, the needle is withdrawn and

digital pressure applied over the puncture which is then sealed. A smear is then made on a slide and NNN tube inoculated. It is only rarely that there is failure to obtain blood and this is usually due to the needle being too short (Napier).

Tibia puncture is made in case of young children (where the sternum is soft) in the middle of the shaft with the sternal puncture needle. The percentage of positive results is smaller than with sternal puncture.

Formo-gel or aldehyde reaction.

For the Formol-gel or aldehyde reaction 40 per cent formaldehyde is added to 1 ccm of suspected serum in a test tube. The mixture is then shaken, in a few minutes the serum will solidify and then becomes opaque, the degree of opacity being the criterion for the test being positive; the test should be read after 24 hours. In early stages of the disease, the test is negative and it gradually disappears progressively during convalescence. The test is negative in cutaneous leishmaniasis. Partial solidification may occur in tuberculosis, leprosy, and syphilis and chronic malaria, but serum does not become opaque.

Chopra's antimony test (serum test). Finger prick blood test

Whole serum and serum diluted 1 in 10 with distilled water are put in miniature test tubes ($2\frac{1}{2}$ to 3 inches long, made by sealing one end of a piece of glass tubing 4 to 5 mm in diameter), with a capillary pipette. A 4 per cent solution of urea-stibamine made with distilled water is then slowly run along the side of the tubes. A heavy coarsely-flocculent precipitate forms when the antimony solution comes in contact with the serum. Tartar emetic does not give this reaction but stibosan does. In very early cases, 1 in 10 dilution of the serum may give negative results and whole serum should be employed. A correct diagnosis can be made by this test in 88.2 per cent of cases as compared with 83.5 per cent of the aldehyde test.

Chopra reaction.

For the Chopra reaction a drop of blood from the cleaned finger is received into a small test tube ($\frac{3}{8}$ inch internal diameter and 2 inches long) containing 0.25 ccm of a 2 per cent solution of potassium oxalate. The corpuscles are allowed to settle down and the test is performed with the supernatent fluid. A 4 per cent solution of urea-stibamine is added in exactly the same way as in the serum test. A flocculent precipitate means a positive test. The reading should be taken from 5 to 10 minutes after mixing the solution. A positive test is obtained in 86.4 per cent of cases. Both these tests become negative when the patient is cured.

According to Schmidt and Peter, the Chopra's reaction with urea-stibamine or neostibosan is a method of diagnosing Kala-azar, which is suitable for practical conditions. In cutaneous leishmaniasis the reaction seems to be of little specificity. The pentavalent antimony preparation, solustibosan, did not give the Chopra's test, proving that the therapeutic efficacy is in no sense dependent on the precipitation.

Mix one part of serum in two parts of distilled water. A turbidity and later a flocculent precipitate develops in positive cases. If water is poured on the surface of the serum a ring effect is produced. This test is due to a marked increase in the euglobulin content of the serum.

Diagnosis of post-Kala-azar dermal leishmanoid is easily made by the typical hypopigmented macules and the erythematous rash. A nodule snipped off with a pair of scissors and a smear on a slide is made by rubbing the cut surface; on staining parasites are found in the endothelial cells or in a free condition in positive cases.

Prophylaxis.

In India no animal reservoir of infection has been found and man is always the source. Once treatment is begun the parasites disappear from the peripheral blood. The treatment should, therefore, be taken on a mass scale. Patients with dermal leishmaniasis are potential sources of danger. Sandflies should be prevented from breeding. The control of sandflies in rural area is very difficult. Paving of floors helps; holes, cracks or fissures are sprayed with kerosine oil emulsion. It has been shown that sand flies prefer bovine to human blood, but is doubtful if proximity of cowsheds, etc., will attract these and save man. Sandflies are sensitive to smoke and their range of flight is short; they do not breed in the open and their larvae could be destroyed by spraying with some antiseptic in the possible breeding places.

Prognosis.

In untreated cases in India the mortality ranged between 75 to 98 per cent and the mortality in infantile form was the same. The fever lasted for a year

or longer, the high remittent fever being followed by complete absence of fever for a month or two. After a total period of about two years, some cases assumed a chronic form with no fever but with anæmia and enlarged spleen; others ended fatally. Death is caused by some complication, *e.g.*, dysentery, pneumonia, cancrum oris, etc. In children the disease runs an acute course, death often occuring in about six months. If full treatment with antimony is given between the 3rd and 12th month of disease, 85 to 90 per cent of cases recover. Of the number that relapse, two-third are subsequently cured by proper treatment. If the disease has lasted for more than a year, the patient may become weak and emaciated and is liable to get complications, such as cirrhotic liver, jaundice and ascites, ending fatally.

3. Specific Treatment

(1) Antimony and its Derivatives

Historical.

Antimony has a very interesting history. Grey antimony has been used from the earliest times in the East as an 'eye salve' for protection against endemic eye diseases and as a remedy for oriental sore. In the 16th and the 17th centuries antimony was considered as a panacea for many diseases, *e.g.*, syphilis, leprosy, plague, cancer, ague, perhaps a forecast of its most recent use in chemotherapy. As early as 1631 the preparation, antimony tartrate or *tartar emetic* came into existence and by the end of the 17th century more than a hundred preparations of antimony were in use in medicine, chiefly against plague and other infectious fevers. Owing, however, to its indiscriminate use many deaths occurred and it was discarded to a great extent, and in many places its use was actually prohibited.

The modern development of antimony therapy began in 1907 following the work of Plimmer and Thomson who demonstrated experimentally the trypanocidal action of tartar emetic. The recent development of antimony preparations is due to work of Professor Hans Schmidt who produced an important series of effective compounds, which have proved very useful in the treatment of some of the tropical diseases of parasitic origin, such as trypanosomiasis, leishmaniasis, bilharziasis, etc. The compounds largely used during early days were the double tartrates of potassium and sodium, for the obvious reason that being double salts they are not readily dissociated, and therefore have not such marked irritant or corrosive properties as the inorganic salts. They, however, suffer from certain disadvantages, one of the chief being that small doses, a maximum of 2½ grains, can only be given at a time, and the treatment is very protracted in such diseases as trypanosomiasis, where a high concentration of antimony in the body, maintained for a long time is necessary to produce the desired therapeutic effects. Subcutaneous and intramuscular injections of these compounds are extremely painful, and even when administered in oil, are very unsatisfactory. Intravenous administration, which is a more difficult procedure, is the only method by which they can be given. It is for this reason that efforts have been directed towords preparing such compounds as stibosan, urea-stibamine, neostibosan, antimosan, etc., which can be administered readily in fairly large doses, without producing untoward effects, so that the therapeutic effects are rapidly attained.

(2) Pharmacological Action

The action of the antimony ion in the main resembles that of the arsenic ion, the chief differences lying in its stronger local irritant effects and more difficult absorption.

Action on protozoa. The salts of antimony, arsenic and bismuth have been shown to have special affinity for certain pathogenic protozoa. Antimony destroys trypanosomes in drawn blood in such high dilutions as 1 in 500,000. The non-pathogenic protozoa such as the paramœcium are not affected even by concentrations of 1 in 5,000. It would appear therefore that these compounds have a specific effect against certain pathogenic protozoa in the same way as quinine has against the malarial parasites.

Externally. When rubbed into the skin, antimony salts produce a characteristic inflammation, at first papular then vesicular and lastly pustular. The rash looks like the eruptions of smallpox. Given subcutaneously, the salts give rise to severe pain and inflammation, often followed by suppuration and sloughing.

Internally. When given by the mouth, tartar emetic has an acrid metallic taste and in very small quantities it produces no symptoms except perspiration. Large doses (2 to 3 grains) give rise to nausea and vomiting accompanied by marked depression and the usual accompaniments of emesis, *e.g.,* salivation, profuse perspiration, acceleration of the pulse, etc. All these symptoms are produced reflexly through the medulla. As an emetic the action of tartar emetic is somewhat tardy, taking 8 to 20 minutes and sometimes half an hour.

Absorption and distribution.

In the stomach, antimony produces irritation. As a rule vomiting removes the compound from the stomach and very little passes into the intestines, but when it does it produces diarrhœa and colic. Absorption of antimony is very slow from the skin or from the gastro-intestinal tract. As it is too irritating to be administered by subcutaneous or intramuscular routes, the intravenous route is the only possible one. Fortunately the toxicity of these compounds when given by this route is very low, as large amounts are rapidly excreted by the kidneys. From the blood, antimony passes into the tissues much more slowly than arsenic. It is found in considerable quantities in the liver, kidneys and intestines come next, but contain smaller quantities. The other organs only show small amount of the metal. The volume of both the liver and the spleen is considerably increased after intravenous injections.

The distribution of antimony in the system depends to a large extent on the quantity administered; after gradual administration of small doses, considerable quantities are only found in the liver, which stores antimony and in the kidneys which excrete it. Like arsenic, antimony gradually accumulates in the hair and nails.

Circulation.

The cardio-vascular effects produced by antimony resemble those produced by arsenic, but acceleration of the pulse seen after tartar emetic is a reflex effect due to the emetic action and not to the absorption of the antimony ions. When injected into a vein, antimony acts directly on the cardiac muscle and capillaries, producing a slow and weak pulse; the heart muscle is depressed, the capillaries are dilated. There is paralysis of the walls of the arterioles due to its direct action on the muscular coat; the vessels of the splanchnic area are particularly affected. The blood pressure falls on account of vaso-motor paralysis; tachycardia may occur and may be distressing. The heart becomes weak and rapid at first, and is later slowed, the contractility of the cardiac muscle is eventually destroyed and the heart stops in diastole.

The alkalinity of blood and the number of erythrocytes are said to decrease while the leucocytes are said to be increased. Tartar emetic and organic antimony compounds do not hæmolyse or agglutinate erythrocytes either *in vitro* or *in vivo*. In maximum doses it produces leucopænia in the peripheral blood and increased phagocytosis in the spleen.

After the injection of trivalent antimony preparations, bradycardia of short duration setting in immediately after injection and a prolonged retardation of the pulse, which gradually develops in the course of the injection series, have repeatedly been reported.

Respiration.

There is slight initial acceleration but soon there is nausea and the respiration becomes shallow and irregular. In cases of poisoning, respiration is very slow, laboured and finally ceases along with the stoppage of the heart. Intravenous injections stimulate the respiration slightly; large doses produce marked congestion and œdema of the lungs, in fatal cases of poisoning.

Secretions.

The secretions such as perspiration, saliva, mucus, sputum, bile, etc., are increased. The quantity of urine is increased with small doses; with large doses it is decreased and may even be suppressed.

Metabolism and temperature.

The effects of antimony on metabolism have not been studied so thoroughly as those of arsenic, but antimony compounds seem to present a strong resemblance to the arsenicals. Prolonged use of toxic doses produces fatty degeneration of many organs, probably owing to diminished oxygenation and metabolism. Antimony salts have a distinct antipyretic effect. Vomiting is also accompanied by a fall of temperature which may amount to 1°C (1.5° to 2°F.).

Central nervous system.

In the frog, subcutaneous injections of the double tartrates produce initial stimulation of the medulla followed by paralysis. Later, the spinal cord and the motor ganglia of the brain become paralysed and the reflexes are lost. It has a depressing effect on the nerve cells.

Excretion.

Antimony salts are mainly excreted by the stomach, the intestines and the kidneys; traces occur in the bile, the sweat and in the milk, and possibly also in the bronchial secretion. There is less danger of cumulative poisoning than with arsenic, since antimony is more rapidly excreted. After intravenous injection of 0.12 gm of tartar emetic, 30 per cent of antimony is excreted in the urine in 48 hours of 1.7 gm injected in a six weeks' course, 1.0 gm was recovered from the urine. After a course of neo-stibosan antimony disappeared from urine in 6 to 12 days. No data are available as regards the quantity excreted by the fæces. It should be used with caution in diseased conditions of the kidneys, the alimentary, the circulatory and the respiratory tracts, especially the former. Large doses produce inflammation of the kidneys accompanied by albuminuria, anuria, and hæmorrhages in the kidney substance.

Much of the antimony is excreted in the case of the organic compound, by the kidneys within a few hours of administration, and a large proportion of the pentavalent compounds remain pentavalent, only a part being reduced to the trivalent state in the body.

(3) Action of Organic Antimony Compounds

The pentavalent organic antimony compounds all contain antimony in strong linkage. They have no local irritant effect and only cause slight toxic effects; they are much less toxic than trivalent antimony compounds. Their effects on the circulation are similar to those of the stable trivalent compounds.

Chopra (1927) showed that the action of the organic compounds of antimony, such as urea-stibamine, is in the main the same as that of the antimonyl tartrates. Intravenous injections of most of these compounds produce a fall of blood pressure which is often more marked than in the case of tartrates. The heart becomes slow and irregular, but it gradually recovers and the blood pressure comes to its normal level. All these compounds have a depressant action on the heart, and relax the ventricles in the same way as cinchona alkaloids. There is a marked rise of pulmonary pressure. The arsenical compounds such as salvarsan also cause a marked rise of pulmonary pressure. Acute toxic symptoms resembling 'nitritoid crisis' are rare with the tartrates, but they are not uncommon when organic compounds are administered.

On the spleen and the liver the effect of these compounds is noteworthy. In experimental animals there is a well-marked increase in the volume of the spleen accompanied by increase of rhythmic movements. The same is the case with the liver though the effect is not so apparent. The effects produced are more marked in case of the organic compounds than with the antimonyl tartrates. After injections of antimony compounds in human subjects, the patients often complain of a feeling of pain and a sensation of distention in the splenic region, and there may also be uneasiness in the hepatic region. These can be accounted for by the increase in the volume and rhythmic movements of these organs. It is possible that this increase has something to do with the therapeutic effects produced by these compounds. The spleen and the liver act as reservoirs of parasites and the influx of blood charged with antimony into these organs may contribute towards the destruction or expulsion of the parasites from these hiding places. It has been found that soon after injections of an antimony compound, Leishman-donovan bodies make their appearance in the peripheral blood where they were previously absent, showing that the action is of a provocative nature.

Antimony has a stimulating effect on the adrenals. It has been shown by Chopra and his collaborators (1928) that the residual epinephrine contents of the supra-renal glands of rabbits, who have had a course of antimony compounds, is higher than those of normal animals.

Tolerance to arsenic does not confer tolerance to antimony. Some of the infusoria become resistant to the action of antimony.

Toxicity.

The maximum tolerated dose of tartar emetic for rats, rabbits and guinea-pigs by slow intravenous injection of a 1.0 per cent solution is about 0.015 gm per kilo body weight. For a man of 70 kilos (145 lbs.) it corresponds to 1.05 gm; the dose usually given to human beings at one time, *i.e.*, 5 to 10 ccm of a 1.0 per cent solution (0.5 to 0.1 gm) is therefore well within the range of safety. Such a dose would be 10 to 20 times smaller than the maximum tolerated dose and it may be safely repeated. Cases of poisoning are due to increased susceptibility of individuals and deaths have occurred from 2 grain doses given intravenously. It is, therefore, advisable to start with such small doses as 1 to 2 ccm

of a 1.0 per cent tartrate solution, and corresponding doses of other compounds. Repeated doses in animals produce changes in the kidneys and in the liver similar to those produced by the arsenical compounds. Jaundice has not infrequently been noticed in kala-azar patients after a course of injections with antimony compounds and sometimes ascites has followed. The lungs may show hyperæmia and œdema. Marked pathological changes have been seen in guinea-pigs after toxic doses of antimonyl tartrates. These consist chiefly of hæmorrhages in the substance of the internal organs and destruction of the parenchyma of the lungs, kidneys, liver, pituitary and suprarenal glands. Similar changes are produced by the new pentavalent aromatic organic compounds.

Poisoning in man.

The symptoms of acute poisoning resemble those of arsenic; these generally begin with nausea, vomiting and pain in the stomach, which is not relieved by vomiting. Emesis is violent and continuous, and may contain blood. Vomiting is accompanied by profuse diarrhœa, followed by great muscular weakness and collapse. The pulse and the respiration become slow and irregular, the skin is cold and covered with perspiration; cyanosis of the face and extremities is generally marked, and the temperature is subnormal. The patient falls into a comatose condition.

Chronic poisoning is rare and difficult to diagnose. It has been observed in type-setters and is usually mistaken for plumbism. The pentasulphide is used in the manufacture of rubber, and antimony salts are also used in glazing cheap granite ware, and in this way may contaminate food stored in them. Since antimony compounds have come into vogue in the treatment of tropical diseases, symptoms of chronic poisoning are sometimes seen. These symptoms are loss of appetite, depression, headache, giddiness, anæmia, confusion, drowsiness and dimness of vision. The patient complains of a feeling of suffocation or a feeling of spasm of the glottis, discomfort or pain in the region of the stomach, general weakness and exhaustion. Profuse diarrhœa may ensue and ulceration of the small intestines. The blood pressure is low and the blood shows leukopænia and eosinophilia. There may be rapid loss of flesh, dysuria, albuminuria, transient jaundice and paraplegia.

Preparations of Antimony

Like many metals antimony forms two series of compounds, its valency varying from three to five.

Antimony compounds may be classified as:—(1) Inorganic salts. (2) Organic salts, *e.g.*, antimonyl tartrates (trivalent salts). (3) Organic compounds: (*a*) Trivalent; many of these are unstable. (*b*) Pentavalent, these are stable and are commonly used.

(*a*) *Trivalent*

The inorganic compounds are not used in therapeutics. Metallic antimony in fine state of subdivision as an impalpable powder, colloidal antimony and antimony sulphide in a colloidal state, have been used by intramusclar injections in leishmaniasis and other protozoal affections, but their therapeutic action is doubtful.

Antimonyl Tartrates.

The organic salts or the trivalent compounds. Antimonium tartratum or 'tartar emetic' is potasium antimony tartrate contains 36.5 per cent of Sb.

Sodium antimonyl tartrate was originally tried by Thomson; experiments on pigeons and rabbits showed that the sodium salt is less toxic than tartar emetic. The therapeutic effects of this compound are the same as 'tartar emetic'. It is also known as 'stibinol' and contains 38.3 per cent of Sb.

An alkaline solution of sodium antimony tartrate known as 'neostibinol' is less toxic and equally effective.

Organic aromatic Compounds.

Aromatic compounds of antimony. (*a*) Trivalent. A large number of antimony analogues of the organic arsenicals have been prepared. The trivalent compounds, *i.e.*, the analogues of salvarsan are not very stable.

ntimosan.

The highly irritative action and toxic properties of tartar emetic are essentially due to the trivalent antimony only existing in a weak complex combination; the acid solution cannot be neutralised and separates antimony oxide whenever the PH is modified. A stronger complex formation was attained in the potassium antimony III-bis-pyrocatecholdisulphonate of potassium or *antimosan;* it yields a stable neutral solution, which can be injected intramuscularly and which showed a surprising increase in the chemotherapeutic index against trypanosomes.

lco-antimosan Stibophenum; ouadin).

The potassium salt was subsequently replaced in further developments by the sodium salt; sodium antimony III-bispyrocatechol-3:5 sodium disulphonate to which the name of *fouadin* or *neo antimosan* was given. The concentration of the solution is increased and adapted to isotony with the tissue fluids: 1 ccm contains 63 mgm of fouadin substance=8.5 mgm Sb. III, whereas antimosan contained 50 mgm antimosan substance=6.25 mgm Sb III. The increase of the concentration simultaneously produces the advantage of a smaller injection volume. With a pH close to 7, it was possible to adjust the solution for injection to the pH of the tissue fluids; this makes intramuscular injections painless. The antimony oxide used in the production of fouadin is subjected to a special purifying process in order to remove traces of lead, etc. which proved especially harmful with tartar emetic.

The solution of fouadin in ampoules is sensitive to air. A stabilised solution is used in bottles. The drug is sold in ampoules containing 3.5 and 5 ccm (1 cc contains 0.064 gm of fouadin).

Fouadin Concentrate.—The calcium-sodium salt of antimony III-pyrocatechindisulphonic acid, has a peculiarity in that the isotonic solution has a higher concentration (1 ccm=14.3 mgm SbIII) than the sodium salt (fouadin 1 ccm=8.5 mgm SbIV). The injection volume is thereby reduced by two fifths which seemed to be an advantage from the point of view of intramuscular injection. Moreover, pharmacological considerations favoured the introduction of calcium.

On account of the increased antimony content mentioned above, 3 ccm (=42.9 mgm sbIII) of the isotonic solution of the preparation correspond in so far as its antimony content is concerned to the usual full doses of fouadin (5 ccm=42.5 mgm SbIII).

Toxicity tests on mice with intravenous and subcutaneous injections showed that the toxicity in regard to antimony is the same as in fouadin.

Anthiomaline.

Anthiomaline is lithium antimony-thiomalate which contains 16 per cent of antimony. It is prepared as a 6 per cent solution and its dose is from 0.5 to 2.0 ccm (1 ccm=0.01 gm Sb); it is given intramuscularly and a course of 12 to 20 injections is given at the rate of 2 to 3 injections in a week. It has a low toxicity and has been used in the treatment of schistosomiasis granuloma inguinale and leishmaniasis.

Pentavalent. Of the antimonials, the various pentavalent compounds containing p-amino phenyl stibonic acid, *viz.,* urea stibamine and similar urea compounds, neostibosan or diethylamine p-amino phenyl stibonate, and sodium antimony gluconate are the most successful. Triarylstibines were first prepared and later triphenyl-stibamines. The present development of organic antimony derivatives is mostly due to the work done by Professor Schmidt who discovered the process of introducing into the aromatic nucleus the antimonial group, through the agency of the diazo-reaction and most of the antimony analogues of the aromatic arsenical drugs such as atoxyl, salvarsan, etc., were prepared. From aniline by diazo-synthesis it was quite easy to prepare phenyl-stibinic acid and then para-amino-phenyl-stibinic acid (p-stibanilic acid), which is the antimony analogue of p-arsanilic acid. Its sodium salt, sodium para-amino-phenyl-stibinate (or sodium-stibanilate) is the antimony analogue of the arsenical compound known as 'atoxyl' or 'arsamin' (sodium p-arsanilate). This compound was given the name of *stibamine* from its analogy to the corresponding salt of arsenic which is called *arsamin.* A derivative of this, sodium acetyl-p-amino-phenyl stibinate, was called 'stibacetin' which was one of the earlier members of the aromatic series to be used in the treatment of leishmaniasis under the name of stibenyl.

Urea-stibamine.

The sodium salt of p-amino-phenyl-stibinic acid, or sodium p-amino-phenyl-stibinate is also a soluble compound which, though therapeutically active, is unfortunately not very stable. Brahmachari (1922) prepared the carbamide derivative of p-amino-phenyl stibinic acid. This substance, urea stibanilate or ammonium carbamino-stibanilate, called 'urea-stibamine' is a pentavalent compound of antimony and believed to be a substituted urea It has also been prepared and sold under the name of stiburea, urea-stibol, stiburamin.

The combination of urea with p-amino-phenyl-stibinic acid renders the latter compound more stable and soluble (p-amino-phenyl-stibinic acid is not soluble) and at the same time, it is more efficacious therapeutically. It is a well-known fact that when quinine is combined with urea, its solubility and diffusibility are considerably increased, and the resultant compound is able to penetrate better into the tissues; its local anæsthetic action is also much enhanced. Similarly, better penetrability of these compounds probably accounts for the superior therapeutic results obtained by them as compared with the previous compounds.

Stibosan.

Von Heyden introduced a compound, metachloro-para-acetyl-amino-phenyl-stibinate of sodium (chloro-stibacetin or von Heyden 471) under the trade name of 'stibosan'. They later introduced two other compounds diethylamine-para-amino-phenyl-stibinate (von Heyden 693) and neostibosan (von Heyden 693 B). Both these compounds are much more effective than the earlier organic aromatic compounds and are at least as effective as urea-stibamine. Neostibosan is not available in India at present; but it is being prepared in America, so supplies may become available in near futures.

Stibamine has also been combined with glucose and a preparation 'stibamine glucoside' also known as 'neostam' is on the market. A compound p-amino-phenyl-stibinic acid combined with urea and glucose sold under the trade name of 'amino-stiburea'. Most of the antimony compounds in use at present are not very stable in the air, even in a solid condition and in solution some of them change very rapidly.

Table of Preparations of Antimony Compounds

(Modified from "Advances in Therapeutics of Antimony"
By Schmidt, H and Peter, F. M. 1938)

Name	Synonym.	Chemical designations.	Sb Content
Trivalent Preparations			
1. Tartar emetic.	Tartarus stibiatus, Tartarus emeticus.	Antimony tartrate of potassium.	36.5 per cent.
2. Sodium antimony tartrate	Stibyal, Stibnal, Neostibnal, S. A. T.	Antimony tartrate of sodium.	38.3 per cent.
3. (Old-) Antimosan	Potassium Antimosan 661	Antimony pyrocatechin disulphonate of potassium (D 12.5 % Sb)	D) 1 ccm=6.25 mgm.
4. Fouadin (Neo-antimosan)	Sodium antimosan Sdt. 91	Antimony pyrocatechin disulphate of sodium (D 13.5 % Sb)	D) 1 ccm=8.5 mgm.
5. Anthiomaline	110 L	Antimony thiomalate of lithium	D) 1 ccm=10 mgm.

Pentavalent Preparations

1. Stibenyl	Stibacetin	p-acetyl-amino-phenyl-stibinate of sodium.	D) 33 per cent
2. Urea stibamine	Ureastibol, Stiburea	p-carbamino-phenyl-stibinate of ammonia (?)	About 35 per cent (?) (Data & results vary)
3. Stibosan	471	m-chlor-p-acetyl-amino-phenyl-stibinate of sodium.	D) 31 per cent
4. Neostam	Stibamine-glucoside	nitrogen glucoside of p-amino-phenylstibinate of sodium.	About 30 per cent
5. Amino-stiburea	Urea stibamine-glucose	p-amino-phenylstibinic acid combined with urea and glucose.	24.8 per cent
6. Neostibosan	693 B	diethyl p-aminophenyl-stibinate of diethyl-amine.	D) 42 per cent
7. Solustibosan	Sdt 561	Antimony hexonate.	1 ccm=20 mgm Sb

D) denotes that in these cases the antimony content is that stated by the manufacturers whereas the remaining data are obtained from literature.

Table of Toxicity of Antimony Compounds

Name.	Minimum lethal dose in mgm. per kilo. of mice.	Maximum tolerated dose in mgm. per kilo. of mice.	Percentage of antimony.
Potassium antimony tartrate	16	.....	.....
Sodium antimony tartrate	20	.....	.....
Stibacetin and stibenyl	133	.....	33
Urea-stibamine, Stiburea, Ureastibol	250	175	20—43
Stibosan or von Heyden 471	275	200	31
Stibamine glucoside. Neostam	500	300	30
Aminostiburea	250	.....	24.8
Von Heyden 693 & 693 B. Neostibosan	350	250	40

The pentavalent compounds can now be therapeutically tested on the striped Chinese hamster *Cricetulus griseus* or the European hamster *Cricetulus frumentarius* both of which can be easily infected with leishmania. The chemotherapeutic index of some of the compounds is as follows:—

Antimosan 1 : 5, Stibosan 1 : 5 to 1 : 7 and Neostibosan 1 : 50

4. Antimony in Kala-azar or Visceral Leishmaniasis

Before the introduction of intravenous injections of antimony compounds the disease ran a course unaffected by treatment. Many remedies including large doses of quinine, vaccines, etc., were tried but proved useless. Whatever cures were produced were of a spontaneous nature and these were estimated as high as 25 per cent. A concurrent attack of pneumonia or cancrum oris may sometimes produce a cure. Injections of drugs, which produce leucocytosis were tried.

The treatment of kala-azar is now carried out with two classes of antimony preparations.

Trivalent compounds.

Patrick Manson originally suggested the use of antimony salts in kala-azar and Vianna (1913) was the first to try intravenous injections of tartar emetic, and successfully treated American forms of cutaneous and mucocutaneous leishmaniasis. Di Cristina and Caronia (1915) used this salt and successfully treated visceral forms of leishmaniasis in children, prevalent on the shores of the Mediterranean, by intravenous injections of tartar emetic. In the same year Rogers and Muir took up the treatment of Indian kala-azar with this drug and confirmed the above results. Rogers at first used a 1 per cent solution, but later he found that a 2 per cent solution was quite safe and more convenient. Solutions as strong as 5 per cent can be given intravenously but they are apt to produce cough and retching, and it is not therefore advisable to use solutions stronger than 2 per cent. The solutions may be made in distilled water with or without the addition of 0.85 per cent of sodium chloride. It is important to use freshly prepared solutions, as moulds are apt to grow and produce toxins. Solutions can however be kept for weeks if 0.25 per cent of carbolic acid is added at the time of preparation.

Tartar emetic.

Injections were given every second or third day into the veins of the arm the doses being gradually increased from 2 to 5 ccm of a 2 per cent solution, keeping a watch whether nausea and gastric symptoms develop. Knowles (1920) treated many cases in Assam, checking his results by spleen puncture and culture of the material for the presence of *L. donovani*. The effects were marvellous and after a few injections the fever subsided. In some cases rigors followed larger doses, but ceased after a time and the temperature remained at a low level or was normal; the weight of the patient steadily increased and the spleen diminished in size. On puncture of the spleen the number of parasites was found to be reduced; the blood showed a marked increase in leucocytes.

Standard course.

The antimonyl tartrates are dangerous if given in doses larger than the patient can bear; the dose should be increased very gradually. For an average adult, a primary dose of 2 ccm of a 2 per cent solution (0.04 gm of the salt), increased on each occasion by 1 ccm up to a maximum of 5 ccm (0.1 gm) is recommended. Subsequently 5 ccm are given on each occasion. As a general rule it may be stated that a maximum dose of 60 grains (4 gm) per 100 lbs of body weight is necessary to produce a cure.

For debilitated patients the initial dose should be 0.5 ccm increased by 0.5 ccm up to the maximum of 5 ccm. For a child of 3 years it is advisable to begin with 0.5 ccm making 2 ccm the maximum single dose. For children of 12 the maximum is 3.5 ccm the doses for intermediate ages being in proportion. The dose calculated according to pounds of body weight is proportionately larger in children than in adults. The injections are given on alternate days throughout the course; they should be given at least twice a week. A routine course of 30 injections is given and a spleen or liver puncture is then done and 2 or 3 N.N.N. tubes inoculated. If no flagellates develop, and the patient's general condition and blood counts are satisfactory, he is considered cured; otherwise a further course of 10 to 15 injections is given and the puncture repeated.

The maximum dose can also be modified according to the general improvement in the condition of the patient, the chief factors to be taken into consideration

are fever, the size of the liver and the spleen, and the blood picture. If the body weight has increased, the spleen is redcced to the level of the costal arch or at least by 4 inches, the white cell count is above 6,000 and the temperature is normal by the seventh injection, the full course of 4 gm may be modified to 30 injections (2.88 gm). If the temperature falls to normal by the tenth injection, 35 injections should be given; and if between the tenth and sixteenth injection, 40 injections are necessary. If this procedure is followed the cure rate is about 80 per cent.

Tolerance. Tolerance is quickly established, and then much larger doses can be given with corresponding beneficial therapeutic effects. In increasing the dosage, the development of tolerance should be judged from the amount of fever, cough and nausea produced. These should not be allowed to be more than slight; in fact, it may be laid down that nausea is an indication for not increasing the dose. The other important considerations are the state of the pulse, lungs and bowels and in serious cases of disease where emaciation is marked these should be very carefully examined.

Toxic effects. Among kala-azar patients the complications usually met with are diarrhœa, low blood pressure, broncho-pneumonia and sloughing of the connective tissue in the form of cancrum oris. Antimony salts if used injudiciously, accentuate all these conditions, and therefore, great caution is necessary in their administration in such cases. It is of the utmost importance that when treatment is once started it should not be interrupted. Slight diarrhœa, œdema of the feet, bleeding from the gums, traces of albumin in the urine are not contraindications to treatment, in fact, they demand it. Heart failure is the most serious of all the symptoms and cardiac stimulants should be freely used if necessary. Intramuscular injections of 0.5 ccm to 1 ccm of adrenalin are useful in counteracting the vasodilator effect and fall in blood pressure.

If hæmorrhagic lesions occur give calcium lactate, 10 grains three times a day or 5 to 10 ccm of a 10 per cent solution of calcium chloride intravenously. If œdema of the feet becomes evident the doses should be regulated with utmost caution. The treatment is continued till the temperature becomes normal. The fever does not as a rule subside before ten injections have been given. In a few cases the temperature may fall after 3 or 4 injections; in others it may keep above normal till 40 injections have been given. A few cases show a slight rise of temperature after each injection. In some cases fever and rigors occur after the first injection. This is believed to be due to the destruction of parasites by the compound, as such reactions do not occur in patients who are not suffering from kala-azar, and they become less and less marked as the patients improve.

For the next five years after the introduction of antimonyl tartrates little further progress was made in the treatment of the disease in India. Sodium salts were substituted for the potassium salts and a number of purified brands of the antimonyl tartrates suitable for intravenous injection were put on the market. The greater solubility of sodium antimony tartrate makes it possible to prepare a scale form of the salt. As the purity of the salt is essential for the preparation of the scale form, it gives the practitioner greater security. With scale preparations the severe reactions met with in the early days of treatment were minimised. A larger number of other antimonyl tartrates were prepared but they proved to be no more effective than the antimonyl tartrates of sodium and potassium.

(1) Pentavalent Compounds

The introduction of these pentavalent compounds of antimony was an important advance in the treatment of kala-azar and marked the second phase in the treatment of leishmaniasis with antimonials. The advantages claimed for the pentavalent aromatic compounds according to Napier (1927) are:—

Advantages.

(1) These compounds are much less toxic, and can therefore be administered in larger doses. The minimum lethal dose is 15—25 mgm per kilo in mice in the case of the trivalent compounds, whereas it is 200 to 500 mgm in the case of the pentavalent compounds.

(2) The total amount of antimony which is necessary to effect a cure can be administered in a much smaller number of doses. About ten doses of the pentavalet compounds against thirty doses of sodium antimony tartrate can be looked upon as the average number necessary for an ordinary case. This reduction in the number of injections means a reduction in the time the patient is under treatment from two months to about three weeks, which in its turn means that a greater percentage of the dispensary class of patients will complete a full course of treatment.

(3) Resistant cases which respond slowly when treated with the tartrates, improve rapidly with larger doses of antimony in the form of the less toxic pentavalent compounds. On the other hand certain cases which fail to respond to pentavalent compounds have been cured by injections of tartrates.

(4) Certain disagreeable symptoms, such as coughing and severe joint pains, which are frequently associated with the tartrate treatment, do not occur when the pentavalent compounds are used. In a number of cases these symptoms are so severe that they necessitate the reduction of the dosage to such an extent as to prolong the course of treatment almost indefinitely and reduce considerably the chances of an eventual cure.

(5) The death rate amongst kala-azar patients under treatment has been very markedly reduced since the introduction of the pentavalent compounds. One of the most frequent causes of death during the course of treatment, namely, pneumonia, has been almost entirely eliminated.

Napier (1924) reported a death rate of 14.4 per cent in a series of 139 cases treated with sodium and potassium antimony tartrate. The same author (1926) found the death rate to be 4.2 per cent in a series of 167 treated in the Carmichael Hospital for Tropical Diseases, with six different pentavalent compounds.

Disadvantages.

The disadvantages of pentavalent compounds are comparatively few. The post-treatment jaundice is a little more frequently encountered, but it is not so serious as is the case with organic arsenicals. Anaphylactoid symptoms may suddenly appear towards the end of the course of injections but these are of a mild type. There is only one thing which stands in favour of the antimonyl tartrates and that is the high cost of the pentavalent compounds. Taking 3 gm as the total dose necessary to effect a cure, the cost of curing a patient will be a few annas with tartrates against a few rupees in case of the pentalvalent compounds.

Dosage

Dosage of pentavalent compounds. The initial dose suggested in the case of urea-stibamine and allied compounds is 0.05 gm, increased by 0.25 gm at each

injection up to 0.2 gm. It is better to start with 0.1 gm for the first dose, 0.2 for the second and 0.25 for the subsequent injections. With stibosan and neostibosan an initial dose of 0.2 gm and subsequent doses of 0.3 gm can be given. Children should be given smaller doses but they tolerate proportionately larger doses than adults. Infants of 18 months to 3 years of age will tolerate 0.1 gm, children of 6 years 0.15 gm and children of ten years or over 0.2 gm.

Standard course

The pentavalent compounds of antimony have completely revolutionized the treatment of kala-azar in India and with neostibosan, the toxic effects observed in the case of other preparations, have been reduced to the minimum. The standard scheme in adults consists of 10 injections one every other day; the initial dosage given is 0.2 gm followed by 0.3 gm. In the case of children relatively larger individual doses are required. It was found possible to reduce the duration of treatment by means of the concentrated course (0.2 gm followed by 7 doses of 0.3 gm each per day) to 8 days, and by means of the hyper-concentrated course (0.3 gm, 0.4, 0.5, 0.6 gm) even to 5 days. The 8 days' treatment appears to constitute the optimum and even with this toxic symptoms may appear.

If the original course lasting for 3 weeks is given, clinical improvement occurs steadily during the course of the treatment. The fever comes down usually after the 4th to 5th injection and the volume of the spleen is reduced. A characteristic feature is the rapid return of the leucocytes to normal and there may even be leucocytosis; the eosinophiles may show a marked increase; the red corpuscles count approaches normal.

Total dosage

In the intensive treatment, the clinical symptoms are often influenced to a marked degree only at the end of the course; the curative process continues as a result of the after effects of the treatment. The best guarantee for successful treatment is said to lie in the total dosage administered, which is 3.0 gm per 100 lbs of body weight for mass treatment. The determination as to whether the cure is permanent or not is furnished by an examination made 6 months later. The fact that with the standard treatment a very high percentage of patients can be cured and that intensive treatments or repeated treatments are only rarely necessary, is of great importance in combating kala-azar in India.

The rapid excretion of the major part of the pentavalent preparations of antimony as compared with tartar emetic—subsequent to therapeutic doses, shows that no cumulation need be anticipated during the treatment even if this is greatly intensified.

Most of the compounds are supplied in sealed ampoules containing the dose required. The top of the ampoule is broken off and the contents are poured into a sterile test tube containing 3 ccm of sterile distilled water; not less than 3 ccm should be used for 0.2 gm of the drug. This is sucked up in a sterilised syringe, and injected in the usual way. If ampoules are not available the drug is weighed on a clean piece of paper. The solutions should be prepared at the time of the injection whenever possible, but they can be kept for a few hours.

There are several brands of this product on the market in India besides urea-stibamine (Brahmachari); 'stiburea', 'urea-stibol', 'stiburamin', have more or less the same composition and action. Brahmachari calls it ammonium carbamino-stibanilate or urea-p-stibanilate, but it has been shown by the author and his co-workers (1928) that it is not a compound but a loose combination of urea with para-amino-phenyl stibinic acid. It was found that urea can be completely separated from the compound by repeated washing with alcohol. Besides the variation in the composition of different specimens on the market, even those prepared by the same firm vary enormously. The antimony content shows a variation from 20 to 43 per cent. Ureastibamine is a brown amorphous powder soluble in water forming

a clear yellow solution. Its toxicity is very low. The initial dose is 0.1 gm increased by 0.05 gm up to a maximum of 0.25 gm given twice weekly. The patients quickly develop tolerance to the drug and its action in restoring the normal leucocyte count is rapid and most beneficial. The average amount of the drug necessary for the cure of a case is 2.6 gm, the average number of injections is 10 to 12, and days of treatment 32 as compared with 30 to 90 injections and a total period of nearly three months in the case of antimonyl tartrates. The author has successfully treated patients suffering from kala-azar with daily injections of urea-stibamine for 6 to 8 days, the first dose being 0.25 gm, second 0.3, third and subsequent doses 0.55, a total of 2.5 to 3.0 gm being given according to the weight of the individual. The duration of treatment has thus been reduced to less than ten days. The earlier a case is treated the more effective the treatment.

With higher doses, hæmorrhages from the gums, nose and stomach sometime occur and even retinal hæmorrhages and collapse have been observed. The solution of the drug should not be boiled and the solid drug should not be kept exposed to air.

Chemically amino-stiburia is p-aminophenylstibinic acid combined with urea and glucose. The addition of glucose is said to add to the stability and to the diffusibility of the drug. This compound is quite effective against leishmaniasis. The total quantity required to produce a cure was 3.35 gm per 100 lbs., that is, about the same as other pentavalent aromatic compounds. It is useful in resistant cases of kala-azar.

Stibosan (von Heyden 471).

Von Heyden 471 is chemically metachlor-para-acetylaminophenylstibiate of sodium. It is a light brown powder, non-hygroscopic and does not decompose in an ordinary corked bottle at room temperature. It is readily soluble in distilled water and forms a sterile solution which does not decompose readily.

Stibamine glucoside or Neostam.

Neostam was prepared by Henry of the Wellcome Research Laboratory. Napier (1929) treated 57 cases with this drug with good results. The compound is easily soluble in distilled water making a 4 per cent solution and is injected intravenously in doses of 0.05 to 0.3 gm. An initial dose of 0.1 gm and a maximum dose of 0.2 gm are satisfactory dosage for patients of average weight. The injections are given on alternate days. The total curative dose is 2 to 3 gm. The drug is well tolerated in comparatively large doses.

Von Heyden 693 or Neostibosan.

Neo-stibosan is diethyl para-aminophenylstibinate. Napier tried neo-stibosan in a series of 61 cases without any deaths; fifty were cured, 2 failed to respond to treatment; the mean dose was 3.35 gm per 100 lbs body weight. It gave the lowest death rate and proved to be superior to most of the pentavalent compounds now used. Ten injections produce a complete cure, the total quantity required being 3 to 4 gm. This is a stable compound and has good keeping properties. The anaphylactoid symptoms as well as jaundice are less common with this drug. There were no disagreeable symptoms, with the exception of vomiting.

Neostibosan B.

Neo-stibosan B is of the same composition as the above but prepared differently; it was introduced to avoid vomiting. This compound forms an isotonic solution in 25 per cent concentration and can be given effectively intramuscularly but intravenous route is preferable in adults; 0.3 gm can be given by daily injection and 8 injections *i.e.*, a total of 2.4 gm suffice to cure the Indian disease. But in Sudan, in China and in the Mediterranean area larger doses are required. There is entire absence of nausea and vomiting and the patients tolerate the maximum dose from the very first injection. The spleen should reduce considerably after 3 weeks and patients gain weight. In some cases progress is slow. Any sharp rise of temperature is probably due to latent malaria. In relapsing cases the temperature mounts slowly. Patients afebrile at the beginning of the treatment commonly show febrile reaction daily or a sharp rise after each injection.

Solustibosan.

One disadvantage of neo-stibosan is that when dissolved in water it does not make a very stable solution. This drawback has been overcome by a new pentavalent antimony compound 'solustibosan'. It is probably a derivative of a hexonic acid containing pentavalent antimony. It is a clear colourless solution which does not give the serum precipitation test. The metallic content is 20 mgm of metallic antimony per 1 ccm thus corresponding closely with 1 ccm of a 5 per

cent solution of neostibosan which contains 21 mgm of antimony. It is stated to be less toxic than neostibosan; 18.5 ccm per kilo of body weight is lethal dose for a mouse, as against 6 ccm of a 5 per cent neostibosan solution. The drug may be given subcutaneously, intravenously or intramuscularly.

Excretion takes place exceptionally quickly. On the first day about 80 per cent of the injected Sb appeared in the urine and 1/4 per cent in the faeces. After 48 hours only traces are found in the excretions. The good general tolerability of solustibosan is thus due to its rapid excretion. Repeated large doses of antimony can, therefore, be given at short intervals without producing cumulative effect. With neo-stibosan approximately 50 per cent was found in 24 hours in the urine.

The drug is in the same class as neostibosan and can be given intramuscularly.

Relative value.

Relative value of pentavalent compounds in the treatment of kala-azar. The sterilising dose per kilo body weight of patients works out to be 0.054 and 0.06 gm for urea-stibamine and stibosan respectively, the curative value is about the same. The number of injections in the case of all pentavalent compunds was nearly equal but was lowest with neostibosan. The average number of injections before the fever subsided was as follows:—Stibosan (No. 471) 5.6; stibamin glucoside 5.4; urea-stibamine 5.1; neo-stibosan (No. 693) 4.57; aminostiburea 4.48. Stibosan and neostibosan are the most stable compounds. Neostibosan is the most inocuous and produces a cure in the shortest period; it gives the lowest death rate during treatment. Patients can now be cured with a routine treatment of eight daily injections of 0.3 gm of neostibosan.

Disease under treatment.

The course of the disease under treatment. The fever usually subsides after the fifth injection, or even earlier but it may persist for a fortnight or more. In most cases the downward tendency of the temperature is immediately apparent, in some cases the temperature remains at about 100°F throughout the course of injections only coming to normal when the course is discontinued. In some patients a reactionary rise after each injection is observed. A sudden sharp reaction means that the dose has been too big.

The general condition of the patient begins to improve soon after the injections are started. The weight may decrease slightly at first but then rapidly increases during the course. The hair ceases to fall out and regains its normal lustre; the appetite improves and there is a sense of well-being. In women menstruation usually begins when the course of treatment is ended. The spleen diminishes rapidly in size and in most cases disappears below the costal margin at the conclusion of the treatment. In some patients the effect on the spleen is slower and in others it is only appreciable when the course of injections is nearing completion. The behaviour of the spleen is dependent on its consistency; when it is fibrous and hard, diminution beyond a certain limit is not possible. The liver seldom shows any tendency to decrease until after the injections have been completed.

The blood picture improves rapidly and by the time the course is completed the leucocyte count is normal or may even indicate leucocytosis; there is a noticeable increase in the eosinophiles. The erythrocyte count also shows an increase.

No dose, however large, can be guaranteed to produce a 100 per cent cure rate. A spleen or liver puncture gives the best indication of cure but it should be remembered that the effect of antimony continues for some time after the

last injection. This is shown by the fact that patients who show Leishman-donovan bodies on spleen or liver puncture become entirely negative some weeks later. The best plan is to give a full course of injections and if there is a relapse a more intensive second course should be started.

Relapses.

If the full amount of the drug has been given relapses are not very common; they are only frequent after insufficient treatment. There is no evidence to show that patients who have had previous injections of antimony require much bigger doses to eradicate the disease in the event of a relapse, but the disease in some cases is much more resistant and these require larger doses. When a relapse occurs, the patient should be given a more prolonged course of treatment. A definite enlargement of the spleen usually accompanies a real relapse as distinguished from other forms of fever. In relapses after antimony tartrates one of the pentavalent compounds should be tried.

(2) Antimony Resistance

Resistant cases.

Kala-azar patients are divided into three different categories: (*a*) patients who react rapidly, the types of leishmania are sensitive to antimony; (*b*) patients who react slowly; and (*c*) resistant cases due partly to incomplete, irregular and interrupted treatment and partly in some instances to a primarily resistant form of leishmania. Generally speaking, Mediterranean kala-azar is more difficult to influence than Indian kala-azar, and in consequence, larger doses should be given. The preparation of choice is neostibosan of which a single dose in obstinate cases should be increased to 0.6 gm and the injections made in rapid sequence. Unnecessarily long intervals between injections might give rise to antimony resistance in cases of leishmania. With intensive dosage no cases of kala-azar resistant to neostibosan should occur. The aromatic diamidines are indicated with antimony resistant cases of kala-azar, the cases showing hypersensitive reactions to antimonials and the cases complicated by Pulmonary Tuberculosis. Also one of the compounds viz., pentamidine isethionate is very suitable for the treatment of the cases in which intravenous injections are different. Pentamidine is well tolerated by the intramuscular route in a 10 per cent solution. The aromatic diamidines are contraindicated in the cases with Jaundice, hæmorrhages, ascites or signs of renal failure.

Aromatic diamidines.

Noma in children.

Treatment of Noma complicating kala-azar in children.—Noma is a very serious complication of kala-azar, and neostibosan has been used in its treatment with very successful results in children, sometimes in conjunction with blood transfusion and careful local treatment.

The total dosage given at first was 1.8 gm whch was subsequently increased to 2 to 2.25 gm depending on the age of the patient, the duration of the disease and the condition of the liver and spleen. The initial doses were mostly at the rate of 0.1 gm per day administered intravenously increasing to 0.2 gm per day. The following basic rules for the treatment of kala-azar in children with noma as a complications have been given:—

1. Give early large intravenous blood transfusion before neostibosan treatment is started and repeat thereafter when indicated.

2. Start with full daily doses immediately following the first transfusion. When a total of 1 gm has been given the drug may be discontinued after a few days, if the local and general conditions are not progressing satisfactorily.

3. Careful local treatment is very helpful in keeping the mouth and wound clean.

4. As a prophylactic measure in the prevention of noma every kala-azar patient who has hamoglobin reading below 50 per cent and very low white cell count, should receive

a large blood transfusion before neostibosan treatment is started, and oral hygiene should receive careful attention.

Antimony in Pregnancy.

Antimony compounds in therapeutic doses have no marked effects on the gravid uterus. Treatment therefore should not be withheld in any stage of pregnancy. Many pregnant women treated with antimony go on to full term and give birth to healthy children.

(3) Evidence of Cure

The criteria of immediate clinical cure consists of (*a*) subsidence of fever and maintenance of apyrexia; (*b*) improvement of general health and gain in weight; (*c*) decrease of the size of the spleen; (*d*) increase of leucocyte count to near about the normal level, *i.e.*, about 5 thousand per cmm; and (*e*) well marked improvement of hæmoglobin value. There are really no good criteria for permanent cure of kala-azar. A patient may relapse after fulfilling all the criteria of immediate cure and no parasites being found on sternal puncture after treatment. The best criterion of permanent cure that holds good for 95 per cent of the cases of Indian kala-azar is the freedom of the patient from all symptoms of kala-azar for a period of six months after immediate clinical cure. Relapses after this period are extremely rare. It has been shown that even though cultures from the spleen and liver puncture are positive weeks or months after the completion of treatment, the patient may still attain a cure. During treatment with pentavalent antimony compound the aldehyde and antimony tests become uncertain at first and after the full course of treatment soon become negative.

Lloyd and Paul's test. In normal serum there is on the average 0.16 gm of euglobulin per 100 ccm of serum or approximately 5 per cent of total globulin; in well-established cases of kala-azar it is 1.5 to 2.5 gm per 100 ccm *i.e.*, 40 to 50 per cent of the total globulin. Lloyd, Napier and Paul applied the method of protein graphs to estimate the cure of kala-azar after treatment with antimony compounds. The protein fraction estimations are made by the refractometric method described by Robertson (1924). There is a steady ascent of the pseudo-globulin and a steady descent of euglobulin, and finally euglobulin attains the normal figure of approximately 0.1 gm per 100 ccm of serum when there is complete cure. The graphs give an index of the condition of the disease. It is suggested that high globulin content of serum in kala-azar is an immunity response and such cases react to treatment rapidly, while the low protein type of case with a weak aldehyde test represents the opposite condition.

Chopra and Choudhury's test. By noting the time of gelation with formalin of the sera from the blood of kala-azar patients, it is possible to indicate in a general way, the progress towards cure. Information regarding progress towards cure may be said to be satisfactory, if the time of gelation is more than half an hour. If however the time of gelation before treatment is known, and this can be easily determined from an aldehyde test, the corresponding increase of the time of gel formation also gives useful information.

5. Mode of Action

The mechanism of the curative action of antimony compounds in leishmaniasis and bilharziasis is not understood. The important factor in the treatment of all protozoal diseases, is that when an attempt is made to exterminate these parasites in the tissues, what appears to take place is that the drug destroys the majority of the parasites. The body resistance then rises and the patient's own natural powers of resistance finally eradicate the residual infection. This is almost certainly the case in kala-azar, where not infrequently a patient at the conclusion of a course of treatment may still show a few leishmania in the film on spleen puncture, and yet remain in perfect health thereafter and be cured. The same appears to hold good for malaria and trypanosomiasis, and probably for amœbiasis also.

In vitro experiments show, that tartar emetic has little action on the cultures of leishmania. The toxic effects are not increased by bringing it in contact with fresh animal tissues. The organic arsenicals such as salvarsan, neosalvarsan, etc., are ten times more lethal to leishmania *in vitro* and yet they are not effective in the disease. Antimony never attains such a high concentration in the body as may be directly parasiticidal. The pentavalent compounds do not differ in this respect from the trivalent compounds, and after an injection most of the antimony is, as a rule, eliminated in 3 or 4 days. Some authors consider that pentavalent organic antimony compounds are first reduced to the trivalent state before they become active. It is very probable that the antimony ion in some form constitutes the parasiticidal agent, but whether oxidation or reduction occurs forming new compounds, or whether some protein compounds are formed in the serum, is difficult to say. The mechanism appears to be very similar to that of the action of mercury on the spirochætes, and it is probable that antimony ions act by direct combination with the protoplasm of the parasites, for which they appear to have a special affinity. The readiness with which antimony compounds dissociate into antimony ions is an important factor in their parasitotropic and organotropic properties. In mice infected with dourine, tartar emetic rapidly destroys the trypanosomes, since they disappear from the peripheral blood stream in 2 or 3 hours after the injection. With the pentavalent compounds, there is an interval of from one to two days before the trypanosomes disappear; but whether this latent period is due to a gradual reduction to a trivalent compound is not known. In human kala-azar there is also an interval, which suggests that antimony in some way stimulates certain body functions, rendering the body environment detrimental for the development of leishmania. *Experimental work.*

Chopra and Das Gupta (1927), showed that pentavalent compounds given intravenously, stimulate the rhythmic contractions of the spleen which help to release parasites from the heavily infected cells, while increased functioning of the adrenals, dialates the vessels of the liver and spleen, thereby increasing capillary permeability and diminishing the nutrition of the endothelial cells. If the action of antimony was merely stimulatory, better results would be obtained by repeated small doses. Clinical experience however, shows that best results are obtained by pushing the treatment to the point of physiological limits. It has been suggested that an important factor in producing a cure is the immunity response of the individual patient. *Role of spleen.*

Whatever may be the exact mode of action, there is no doubt that the death rate in kala-azar has been reduced from 90 per cent to 5 per cent, and that in Assam and Bengal, on account of extensive use of the antimonials, the disease no longer exists in epidemic form and may in time completely disappear.

6. Routes of Administration

A five to ten per cent ointment of metallic antimony in lanoline base was recommended in the treatment of leishmaniasis, one drachm being rubbed over the abdomen every second or third day. It has no appreciable effect on the course of the disease. Oriental sores have been successfully treated with a 2 per cent ointment of tartar emetic, the application being made at night. The irritant action of tartar emetic when applied to the skin in the form of an ointment is due to its being broken up by the acid formed by decomposition of the sweat. If sodium bicarbonate is added to the ointment the irritating effects are considerably reduced. *By the skin.*

The antimony salts are not suited for administration by the mouth except in very minute quantities. Their irritant action gives rise to emesis and they are not easily absorbed from the alimentary canal. This route cannot be adopted when it is necessary to introduce large amounts into the body as in cases of protozoal diseases. *By mouth and per rectum.*

Rectal injections of tartar emetic, urea-stibamine, etc., have been tried. The former salt is too irritating and causes much pain and discomfort. The latter is not so irritating but the quantity absorbed by this route is too small to produce a concentration in the blood lethal for leishmania.

Subcutaneous and intramuscular.

Subcutaneous injections produce cellulitis and abscess formation. Urea stibamine and allied compounds (stiburea, amino-stiburea, stiburamine, ureastibol etc.) are administered intravenously in 5 per cent solution in sterile re-distilled water. Neostibosan is administered intravenously in 5 per cent solution and intramuscularly in 25 per cent solution in sterile re-distilled water. Sodium antimony gluconate (stibatin, solustibatin, myostibatin, etc.) as supplied as a ready made solution containing 20 mgm. of antimony per c.c. and also as solution containing 100 mgm of antimony per c. c. is best administered intramuscularly although it can be given by the intravenous route also. The aromatic diamidines, stilbamidine isethionate (M & B 744) and pentamidine isethionate (M & B 800) is administered intramuscularly in 10 per cent solution and intravenously in 1 per cent solution in sterile re-distilled water.

Intramuscular injections even of the double salts are very irritating. Not only is severe pain produced, but extensive necrosis of tissues at the site of injection occurs. Attempts have been made to discover preparations which can be given this way without harmful effects. Such compounds as antimosan, fouadin, stibosan and neostibosan administered by the intramuscular route are non-irritant and quite effective; stibosan and solustibosan are drugs of choice for this. The advantages of this route are that, as compared with the intravenous route, little skill is required. Besides, in very young children it is sometimes impossible to get at the veins embedded in the subcutaneous tissue.

Intravenous route.

By intravenous method uniform success can be claimed, and most of the antimony compounds can be introduced directly into the blood stream with safety. The only objection is that it requires special technical skill, but that can be easily attained with a little practice. Experience has amply proved that the danger of this route is slight if proper care is taken as regards technique and regulation of dosage of these compounds. The technique of intravenous injection is described in the chapter on intravenous therapy.

7. Toxic Effects

A. *Toxic reactions produced by antimony tartrate injections*

Certain toxic symptoms follow injection of antimonyl tartrates. Of all the symptoms cough, metallic taste, and a feeling of tightness in the chest are most frequent. Cough, retching and colic indicate a need for caution. Jaundice, and a larger amount of albumin in the urine than can be accounted for by the disease, are signs of danger. So far as is known acute toxic reactions of the 'nitritoid crisis' type do not occur with antimonyl tartrates. The frequency of toxic symptoms depends partly on the sensitivity of the patient and also on the severity of the disease and length of treatment. The toxic symptoms, if alarming or persistant may call for reduction of dose. If there is persistant quick pulse, severe epigastric pain, persistant vomiting, or reduction in the amount of urine, the course should be suspended. Nephritis and jaundice are a contra-indication to injections. A marked rise of temperature, enlarged and tender lymphatic glands, diarrhoea and necrosis of gums are danger signals. The toxic symptoms that are produced may be grouped as follows:—

Gastro-intestinal and respiratory.

Severe spasmodic coughing may occur immediately after the injection and may be so severe as to end in vomiting. It is an indication that the dose has been excessive and should not be increased. After a large dose the patient may vomit 5 or 6 times. By decreasing the dose and then gradually increasing it, a certain degree of tolerance can be established. Both coughing and vomiting may be induced by giving the injection on a

full stomach or by giving it rapidly. Metallic taste, lack of appetite, a vague discomfort in the abdomen sometime increasing to a convulsive pain, and finally diarrhoea my occur; vomiting may be severe and alarming. Headache, dizziness and fever may be present.

In some cases a violent fit of coughing has followed even the smallest doses, and recurs with each injection. Delayed vomiting may start 4 to 5 hours after injection. Acute diarrhœa may sometimes follow. A metallic taste may be noticed after injection. Codeine ¼ grain, or ten minims of adrenalin given by the mouth a quarter of an hour before the injection will reduce the tendency to vomiting. Pneumonia and lung complications occur frequently with antimonyl tartrates, but they are extremely rare with the pentavalent compounds. If a very large dose is rapidly given the respiration may stop.

Cardiac symptoms.

These consist of cynosis, rapid and irregular pulse, a choking feeling in the chest and tachycardia. Reversible bradycardia with fall of blood pressure, oppressive sensation in the chest and sometime collapse have been noticed towards the end of the course. During the injection there is hardly any fall of blood pressure.

Changes in electrocardiogram may appear in some cases during antimony treatment. The S-T interval as well as the T deflection have been found to alter. The extent of the electrocardiographic changes may be parallel to the degree of chronic bradycardia. The persistent antimony bradycardia differs in its electrocardiographic characteristics from the slowing of the pulse rate due to vagal stimulation. The electrocardiographic abnormalities as well as the bradycardia are therefore considered as resulting from intoxication of the heart muscle. Cases of sudden death during the treatment have occured, several hours after injection and following after physical exertion.

Cerebral symptoms.

These are said to occur more commonly during treatment of trypanosomiasis. Loss of consciousness with incontinence of urine and fæces may sometimes ensue. Severe headache or hemicrania has been observed after 6 to 9 injections and it does not clear up till the injections are discontinued. Herpes zoster may occur.

Arthritic symptoms

Severe joint and muscular pains frequently occur, but are less common towards the end of the course. Pain in one or both shoulders, and lumbago are common complications. The wrist joint, knee joint and ankle joint may be affected, and the pain may last for some time. Usually the joint pains commence 4 to 6 hours after an injection, generally after a total of about 10 grains of antimony tartrate, and they last for about 12 hours. Ten grains of aspirin given half an hour before the pains are expected to begin, diminish their severity. Codeine in 1/6 to 1/4 grain doses counteract most of these symptoms.

Other symptoms.

An irritating papular eruption of the skin may occur on any part of the body. Urticaria (allergic), lymph-adenitis and œdema were rarely observed. Classic symptoms of antimony poisoning, *e.g.*, stomatitis, diarrhœa, anæmia and cachexia may develop. A sharp rise of temperature with rigor is generally due to injection of faulty solution. Profuse sweating or fainting is of rare occurrence.

Fouadin which is also a trivalent preparation, unlike antimonyl tartrates, has no local irritant action and can be given intramuscularly; the shock due to intravenous injection is thus avoided. The general symptoms above described may be absent or much reduced in intensity and frequency. There is less injury to the liver and kidneys.

B. Complications after injections of pentavalent compounds.

Nausea and vomiting.

There is complete absence of asthmatic symptoms peculiar to trivalent compounds. Bronchitis and pneumonia are also absent; no heart or cerebral symptoms were observed. The fit of coughing which not infrequently occurs after injection of antimonyl tartrates does not, as a rule, occur with the organic aromatic compounds. Nausea and vomiting are seen in about 10 per cent of cases, but rarely with neostibosan (von Heyden 693 B) except when the dose is very large. If nausea and vomiting persist, the dose should be reduced. The tendency to vomiting can often be overcome if the dose is increased very gradually. After injections of stibosan, vomiting not infrequently occurs. Generally the vomiting begins within 20 minutes of the injection and it may be preceded by giddiness. If the patient remains quiet in bed, vomiting may be avoided. The injections should not be given immediately after a meal. Diarrhœa may occasionally occur

towards the end of the course and may be severe. The motions are dark, watery and frequent; they contain no blood or mucus. Fatal collapse and death are on record. A severe attack of shivering lasting for 2 hours, may be the only feature. Cardiac, cerebral and arthritic symptoms have not been noticed.

Anaphylactoid reactions.

Symptoms of an anaphylactoid nature resembling the 'nitritoid crisis' (occurring with organic arsenicals) sometimes develop. This is to be expected, as both these series of compounds are of semi-colloidal nature, and give rise to changes in the blood. They also produce an enormous rise of pulmonary pressure. These symptoms set in suddenly after the sixth or seventh injection, when the patient has been receiving maximum doses. Within a few minutes of injection, the face becomes puffy, the voice husky, there may be an urticarial rash on the body, dyspnœa and stertorous breathing. The patient has a feeling of impending death. The pulse becomes weak and imperceptible; the patient is cyanosed, and coma and collapse may soon supervene. The condition though alarming, is rarely fatal, and as a rule all symptoms disappear in two hours. The puffiness of the face may last for as long as 24 hours. These symptoms are more frequently observed with amino-stiburea and urea-stibamine, and are rare with neostibosan. Injections of a few minims of adrenalin or pituitrin, relieve the symptoms. If these symptoms occur, it is best to abandon treatment with a particular compound, recommencing it with minute doses of some other compound. Nausea and vomitting are frequent if the injection is given soon after a meal; consequently the injection are best given two hours or so after the patient has had a cup of milk.

It has already been pointed out that agglutination and hæmolysis of red blood corpuscles does not occur even with concentrated solutions of the antimony compounds. These symptoms therefore cannot be due to embolism of the capillaries of the lungs and other organs. Chopra and his associates (1927), have shown that when organic antimony compounds come in contact with the serum of kala-azar patients, a thick flocculent precipitate is formed. This precipitate is not produced with non-kala-azar serum and this reaction has been used as a diagnostic test for the disease. It is possible that some of the untoward symptoms produced may be due to this precipitation.

Rarely, acute congestion of liver or even symptoms of acute hepatitis are produced by injection of these compounds. The liver becomes enlarged, tender and painful; the temperature rises, and there may be jaundice. The symptoms disappear if the injections are stopped and are apparently due to the toxic action of the compounds on this organ. Jaundice may sometimes appear 1 to 3 months after the course. Dermatitis of an exfoliative nature has not been noticed.

Choice of a compound.

The chief factors to be taken into account in the selection of a compound, are the toxic effects produced, the time necessary to produce a cure, the ease of administration and the relapse rate after treatment. Anaphylactic symptoms are common with urea-stibamine and aminostiburea, but are rare with stibosan and neostibosan. The relapse rate is also lower with the latter compounds and they are more stable. Neostibosan is the easiest to administer as it can be given intramuscularly in doses of 0.1 gm to 0.3 gm without causing pain and it is effective. Both these compounds are, however, expensive. Urea-stibamine and allied compounds are nearly as effective and are slightly cheaper.

Cumulative action. Cautions.

The human body can stand large doses of antimony especially when it is given intravenously. The reason for this is that when administered by this route quantities are eliminated from the system by the kidneys.

Antimony salts should be employed with great caution in weak, anæmic and emaciated subjects. If disease of the heart, kidneys or lungs co-exists special care should be taken. Before starting the injection and during the course of treatment, the urine should be examined frequently for the presence of albumin.

The intravenous administration of the aromatic diamidine is usually associated with fall of blood pressure and the patient may complain of burning sensation all over the body, flushing of the face, giddiness, palpitations, breathlessness, sweating, faintness, etc. Most of these symptoms can be obviated by administering the drug at an extremely slow rate with the patient lying flat in bed or by the previous injection of adrenaline or calcium gluconate. But slow intravenous injection are followed by thrombosis of the vein employed. Intramuscular injection of stilbamidine is very painful but pentamidine is well tolerated by the intramuscular route. No immediate reaction are caused by the intramuscular injections of aromatic diamidines.

8. Summary

The treatment of Kala-azar with antimony compounds is briefly as follows:—

Trivalent compounds.

Sodium antimonyl tartrate is the drug of choice and is given intravenously in two percent solution in normal saline every other day. The first dose is 2 ccm, then 3 ccm, 4 ccm, and 5 ccm, the last being the maximum dose. At least twenty five injections are required. This compound is less effective than pentavalent compounds and is liable to produce reactions, and relapses are frequent.

Pentavalent Compounds.

Neostibosan and Solustibosan are the drugs of choice, but in India urea stibamine is largely used. These drugs are used in 5 per cent solution intravenously and 25 per cent solution for intramuscular administration (only Neostibosan and Solustibosan) in double distilled pyrogen free water.

The initial dose in adults is 0.2 gm increased to 0.3 gm for subsequent injections which are given daily. Usually eight to twelve injections are required. Children get small doses but a child over sixty pounds in weight gets the adult dose. Anaphylactoid reactions (nausea, vomiting, urticaria, dyspnoea) if they occur, should be controlled with five to ten minims of adrenalin given intramuscularly. Anaphylactoid reactions are common with urea stibamine if a course has already been taken with this drug; in such cases the intial doses must be small.

9. Aromatic Diamidines

It was shown in 1937 that diamidines possessed marked trypanocidal activity. This opened up a new field of biological and chemical research. In 1942 the preparation and comparative trypanocidal activity of numerous aromatic diamidines was described. The 4:4'—diamidino derivatives of diphenyl ether, stilbene, diphenoxy propane and pentane were later tried in Leishmaniasis and Trypanosomiasis. The chemical constitution of these compounds may be shown as follows:—

Phenamidine	.....	Am. R.O.R. Am (4:4 diamidino diphenyl ether).
Stilbamidine	.....	Am. R. Ch: Ch. R. Am (4:4'—diamidino stilbene).
Propamidine	.....	Am. R. O. $(CH_2)_3$ O. R. Am (4:4'—diamidino 1:3 diphenoxy propane).
Pentamidine	.....	Am. R. O. $(CH_2)_5$ O. R. Am (4:4' diamidino 1:5 diphenoxy pentane).

Where Am stands for the amidine group $NH_2:C(:NH)$, and R stands for C_6H_4.

The position of substituent amidine group in the benzene nucleus is in each case 4:4'.

Pharmacological Action.—All the four compounds have depressant action on the circulatory system. There is a marked transient fall in blood pressure. The fall of blood pressure is much reduced or prevented by a previous injection of calcium, and could mainly be accounted for by peripheral vasodilatory effect of these compounds. The depressant action of these compounds is only partially antagonized by atropine. The effect of the diamidines on the heart is small and transitory. In general, low concentrations stimulated, and high concentrations depressed the heart. There is, however, no marked deleterious effect. The pressor action of adrenaline is very much reduced by the diamidines.

Respiratory system.

Following slight initial stimulation, the respiration is greatly depressed in intact animals accompanied by dyspnoea which may be due to spasm of the bronchi. On intravenous administration to anaesthetized animals, the main effects observed are increase in depth and volume of respiration. Recovery takes place as soon as blood pressure regains its normal level.

Muscular system.

All the drugs stimulate the plain muscles, such as that of intestine and uterus in high concentrations. These effects were not abolished by atropine. The diamidines have an ergotoxine like action and reduced the action of adrenaline on the uterus, and on perfused vessels of rabbit's ear and cat's hind limb.

Toxicity.

All the four compounds have the same toxicity. The diphenyl ether compound (phenamidine) is less toxic than the stilbene derivative, while the diphenoxy compounds are slightly more toxic.

The administration of the lethal doses depresses the respiration greatly which ultimately becomes very difficult, and violent efforts are made till ultimately the animals lie prostrate and die after a few convulsions. The chronic toxicity experiments also show that there is cumulative toxic reaction.

Therapeutic.

These compounds have during recent years been tried in the treatment of human sleeping sickness and visceral leishmaniasis. While these clinical trials are still in progress in different countries, encouraging results in these two diseases have been reported by many workers.

Treatment with aromatic diamidines is a definite advance and in Sudan and in Calcutta better results have been obtained than formerly with antimony. In antimony resistant cases the results have been very striking.

Stilbamidine and Pentamidine.

Napier has pointed out that these drugs should be used with great care and should preferably be given in antimony resistant cases. Two compounds. Stilbamidine isethionate (M & B 744) and pentamidine isethionate (M & B 300) have been tried. The drugs occur as white powder and are marketed in sealed ampoules. The powder is dissolved in distilled water to make a 1 per cent solution for intravenous and 10 per cent solution for intramuscular injection, the injections should be given slowly, the maximum dose, of stilbamidine for adults, children and infants, being 3 mgm (0.003 gm) per kilo body weight, ¼, ½ and ¾ of the maximum dose for the first, second and third injections. For intramuscular injection maximum dose may be given from the begining of the course of injections. Maximum dose of pentamidine isethionate is 3 to 4 mgm. per kilo for all age groups, for intravenous injection maximum dose is reached in two to three stages, as with stilbamidine, and for intramuscular injection, maximum dose may be given from the begining of the course of injections. The adult dose is 0.025 gm as initial dose; if this gives a reaction, next day the dose given is 0.035 gm but it is preceeded by an injection of 0.25 ccm of 1 in 1,000 adrenaline. If the reaction is mild the next dose of 0.05 gm is given without adrenaline. The doses are increased rapidly by 0.01 gm or 0.02 gm, according to reaction produced, till a maximum dose of 0.003 gm per kilo body weight is reached. Adrenaline is given for the next dose whenever there is a marked reaction to the previous dose and the dose may also be reduced.

In Sudan a much more extended course and a much larger dose has been given—0.75 gm in 24 doses or to 4.4 gm in 70 doses. The good effect of treatment is not immediately apparent and the fever may not disappear in the majority of cases till after the course is completed; exacerbation of symptoms may actually occur after the first few doses.

Toxic reactions and sequelae.

The mild reactions are headache, flushing of the face, sweating and a burning sensation all over the body. In more severe cases, headache is intensive and there is giddiness, faintness, palpitation, epigastric pain and vomiting; collapse and unconsciousness, loss of corneal reflex and incontinance of urine and fæces may occur.

Most of these symptoms can be reduced to minimum by giving a small dose of adrenaline previously and even a moderate dose after the injection will rapidly relieve the symptoms considerably. Vomiting is prevented by giving the drug on an empty stomach, but it is preferable to give it two hours after meal.

Subjective disturbances of sensation over the trigeminal nerve, hyperaesthesia, paraesthesia, anaesthesia, formication, and loss of sense to light touch, but preservation of pressure sensation, may occur. Aneurine in large doses has been prescribed and relief has followed after injections of cobra venom (0.1 to 1.0 ccm of 1 in 100,000 every third day).

Summary:

The aromatic diamidines are very effective in the treatment of Kala-azar and should be tried in all cases resistant to treatment with antimony compounds. These compounds are, however, very toxic and should be used with great care. The drug of choice is 4:4 diamido stilbene, known as stilbamidine which is given intravenously in freshly prepared 1.0 per cent solution in double distilled pyrogen-free water; no dose should exceed one mgm per pound of body weight. The intial dose irrespective of weight is 0.025 gm, increased by 0.01 or 0.02 gm to an individual dose of 0.001 gm (1 mgm) per pound body weight. A minimum of ten injections totaling 0.75 gm for a person of average weight (100 lbs) are required. The course should not be repeated for at least one month.

Post-Kala-azar dermal leishmaniasis does not respond so well to these compounds as to the antimonials.

General Treatment.

Complicating infections such as hookworm, malaria, etc., should be treated. The blood soon returns to normal after the specific treatment, but a course of iron, either ferrous sulphate 9 grains daily or Citrate of ammonium and iron 20 to 30 grains daily is decidedly helpful. Diet should be light as there is a tendency to diarrhoea on full diet. Most patients have good appetite, but at the height of fever high protein diet is not desirable.

Complications should be treated as they arise. Pneumonia is not uncommon. If cancrum oris occurs, the mouth should be kept clean with eusol solution or hydrogen peroxide or boric lotion. It is inadvisable to use strong antiseptics.

10. Treatment of Resistant Cases

Napier defines a resistant case as one who has not been cured by an ordinary course of treatment, which cures 90 to 95 per cent of cases or there is relapse. In such cases there is still evidence of visceral infection. Aromatic diamidines appear

to be the drugs of choice in such cases. This worker has used a prolonged course of 12 to 15 injections with a mean total dose of 0.919 gm and a mean relative dose of 1.048 gm per 100 lbs of body weight and cured 22 gm of 25 antimony resistant cases. For the treatment of stibio-resistant cases, the scheme of treatment is as follows: total dose 2 gm per pound of body weight, are given in consecutive days; rest for 2 to 3 weeks during which subsidiary treatment and any anti-anaemic treatment necessary are carried out. The patient is then examined clinically and haemotologically to see if all the criteria of clinical cure are fulfilled. If it is found that the patient can not be regarded as fully cured a second course of 15 injections will have to be given. In very resistant cases it may be necessary to administer a third course of injections. In Indian kala-azar it has been found that the treatment outlined above is very satisfactory. But cases are on record mostly from the mediteranian area and Sudan in which even such therapy has been followed by relapse. In such cases 'Splenectomy' should be considered in selected drug resistant cases of kalazar and the operations should be followed by further course of chemotherapy.

The course of neostibosan given by turn was:—

"Twelve injections in twelve days, beginning with 0.2, followed by 0.3, 0.4 and subsequently 0.5 gm daily; an interval of twelve days; a second series of twelve injections, beginning with a dose of 0.3 gm; another interval of twelve days; and a third series of twelve injections beginning with 0.3 gm."

"If the patient shows little sign of improvement during the first series of injections, it is advisable to give one or two turpentine injections during the second series. Muir's prescription for the turpentine injections is: 1 part each of turpentine, camphor, and creosote, and 2½ parts of olive oil. Of this, 1 ccm is injected into the gluteal muscle; if a sharp reaction does not occur, a larger dose is injected a day or two later. The object is to produce a very severe local reaction. The turpentine injections are given coincidently with the antimony injections."

"In one of the most resistant cases the writer has ever encountered, a cure was effected by the application of the same principle in a slightly more primitive form by a practitioner of the 'indigenous' system of medicine who placed a vesicatory plaster on the patient's abdomen which caused the whole abdominal wall down to the muscle to slough and left a deep ulcer the size of the palm of the hand; when the ulcer healed the kala-azar was cured."

"When one antimony compound has been used throughout without success, a change to another compound should be tried."

Phenamidine.—A small number of cases have been treated with diphenyl ether derivative of diamidines in India. The observations in this small series of cases show that the immediate clinical cure rate and the final results are inferior to those obtained with the most effective pentavalent antimony compounds or diamidino stilbene.

Usually, two courses of 10 daily injections each, separated by an interval of 10 days are required to produce a clinical cure, the whole course requiring a month. The compound is especially recommended in cases which are sensitive to antimony or are complicated with pulmonary tuberculosis. It is a relatively less toxic compound of the series.

11. The Treatment of Complications

(i) *Cancrum Oris*: Cancrum oris was regarded as the most classical and the most fatal complications of kala-azar. But the use of Penicillin in treatment of this conditions has yielded satisfactory results. The treatment consists essentials of (*a*) Penicillin 20 to 50 thousand units intramuscularly every three hours for 5 to 10 days; local spray with Penicillin solution containing 500 units per ccm every three hours; (*b*) specific treatment being given daily (*c*) adequate nourishment and blood transfusion if required, glucose solution, vitamin B_1 and C and calcium gluconate may be necessary to increase the body resistance. Plastic surgery may be necessary when the general health is restored to normal.

(ii) *Dysentery and severe diarrhoea*: These are relatively serious complications of kala-azar, because the specific treatment is at times not so effective. In the case of bacillary dysentery or severe diarrhœa where no causative organism can be found, sulphaguinidine in 2 to 3 gms doses every four hours is useful. The diet should be restricted to protein hydrolysate, butter milk or peptonised milk, according to the severity of the symptoms. The specific treatment of kala-azar should be the antimonials but the injections should be given in slightly smaller doses and at intervals of 2 to 3 days during the attack of dysentery.

(iii) *Jaundice*: Jaundice is one of the unfavourable complications of kala-azar. It indicates severe hepatic damage and may be associated with severe hæmorrhages. The treatment is symptomatic, *e.g.,* cholagogue mixture, fat poor diet supplemented by methionine and liberal amounts of glucose and vitamins particularly vitamin K parenterally. Specific treatment of kala-azar is with one of the least toxic antimony compounds, viz, sodium antimony gluconate or neostibosan, and the injections are given on alternate days, maximum dose being to be reached very cautiously.

(iv) *Ascitis and probable cirrhosis of the liver*: This is a complication seen mostly in very chronic cases of kala-azar and the outlook is rather unfavourable. The treatment consists of restriction of fluid intake, low fat, high carbohydrate diet supplemented by milk proteins and methionine and vitamins B_1 and C particularly, diuretics, ammonium chloride and mercurial diuretics being used if necessary. The specific treatment is by one of the least toxic antimonials.

(v) *Acute respiratory infections, lober or bronchopneumonia*: Penicillin 25 to 50 thousand units every three hours, is the drug of choice Sulphadiazine or sulphathiazole may also be given, dosage being 4-6 gm. on the first day followed by 4 gm for the subsequent days till the conditions subside. The specific treatment for kala-azar is withheld till the pneumonic or bronchopneumonic conditions subside completely.

(iv) *Kala-azar complicated by Pulmonary or other forms of tuberculous disease*: The combinations of kala-azar and pulmonary tuberculosis was regarded as a fatal one by Napier and others, because of the fact that there may be a fatal flare up of the pulmonary tuberculous focus as a result of antimony treatment of kala-azar. The drug of choice for the treatment of such cases is stilbamidine. The injections are given on alternate days and the total dose is reached in 12 or 15 injections for the cases suffering from non-pulmonary tuberculosis, viz., abdominal or glandular tuberculosis, in addition to kala-azar; stilbamidine or pentamidine should be used for the treatment of kala-azar along with streptomycin therapy for the tuberculous disease. Surgical treatment of tuberculosis is best withheld till the completion of anti kala-azar therapy.

(vii) *Haemorrhage*: Minor haemorrhages such as slight epistaxis, or bleeding from the gums or a few patechaeal hæmorrhage do not require any vigorous treatment, but extensive hæmorrhage, severe purpura with large ecchymoses, bleeding from the mucous membrance recquire antihaemorrhagic treatment by sedatives, coagulants, vitamin C & K, local application of russel viper venom, etc. The specific treatment of kala-azar should be with the pentavalent antimonials and it should be cautiously applied in smaller doses and at two to three days intervals as long as the severe hæmorrhage persist.

(viii) *Agranulocytosis or malignant granulopenia*: This complication is rather uncommon in Indian kala-azar. The treatment consists of the following:—Penicillin 50,000 to 100,000 units intramuscularly every 3 hours in order to combat any septic infection; whole liver extract or pyridoxin either singly or in combination, in an attempt to raise the granulocyte count. Glucose solution with vitamin B_1 and C and calcium intravenously are also given. The specific treatment with one of the least toxic pentavalent antimonials may be given in the previously untreated cases who develop the complications. But in the cases that develop this complications during the specific treatment of kala-azar, it is more rational to withheld antimony treatment till the condition subsides.

(ix) *Severe Anaemia*: In case of severe anæmia, hæmoglobin being 3 to 4 gm per cent, red cell about a million or less it is better to start a blood transfusion along with the specific treatment of kala-azar. Other anti-anaemic treatment as iron and liver extract may also be given along with it.

(x) *General anasarca*: In the vast majority of cases, the œdema is due to lowering of the colloid osmotic pressure, of the plasma caused by the alterations in the blood protein viz., hypo-albuminaemia and hyperglobulinaemia seen in kala-azar. The treatment consists essentially of putting the patient to bed, restricing the fluid intake; the diet should include protein hydrolysates and specific treatment for kala-azar are followed.

12. Treatment of Post-Kala-azar Dermal Leishmaniasis

According to Napier diamino-stilbene is not of much value in this condition, the only specific treatment being with antimony compounds, though cure is affected with difficulty. He has obtained satisfactory results with pentavalent compounds and has used the trivalent compounds, such as antimosan and fouadin in some cases with good results. He recommends the ordinary course used in visceral infection, but injections should be given on alternate days or even more widely spaced. One course is usually sufficient and nodular lesions show signs of improvement, but the hypo-pigmented lesions remain unchanged, regaining their pigment in the course of a month or so. The shrinking of nodules continues for sometime and at least two months should be allowed to elapse before a second course is given.

Injection of a 2 per cent solution of berberine sulphate make the nodules shrink, but this is not considered to be a practical method when lesions are extensive. Large doses of potassium when pushed to its physiological limit may be of value; the nodules may undergo ulceration but heal rapidly after the drug is discontinued.

Treatment of Kala-azar

Summary

Trivalent compounds.

Two per cent solutions of potassium or sodium antimony tartrate are used and injections are given every second or third day. For an average adult, 2 ccm of the solution (0.04 gm of the salt) is the first dose; on each subsequent occasion the dose is increased by 1.0 ccm till a maximum dose of 5 ccm is attained. Afterwards 5 ccm are given on each occasion. Generally sixty grains (4.0 gm) of the salt is required per 100 lbs body weight to affect a cure. For debilitated patients, the initial dose should be 0.5 ccm increased by 0.5 ccm up to a maximum of 5 ccm. For a child of 3 years it is advisable to begin with 0.5 ccm making 2 ccm as the maximum single dose. For children of 12 years the maximum dose is 3.5 ccm; the doses for intermediate ages are in proportion. The injections are usually given on alternate days or at least twice a week thoughout the course and a routine course of 30 injections is given. A spleen or sternal puncture is then made for culture; if no flagellates develop and patient's general condition is good, he is considered cured; otherwise a further course of 10 to 15 injections is given.

Pentavalent compounds.

These compounds have practically entirely replaced the trivalent compounds in the treatment of Kala-azar in India. Neostiban B and urea stibamine are drugs of choice. The standard scheme in adults consists of 10 injections one every other day. The initial dose is 0.2 gm followed by 0.3 gm. In case of children, relatively larger individual doses are required. A total dose of 3.0 gm per 100 lbs body weight of the pentavalent compounds is required to produce a cure.

It is found possible to reduce the duration of treatment to eight to ten days by means of a concentrated course—0.2 gm initial dose followed by seven doses

of 0.3 gm each per day. Some workers give a hyper-concentrated course lasting five days—0.3 gm, 0.4, 0.5, 0.6, 0.6 gm—injections being given daily.

Eight days' course is, however, the optimum and even with this toxic symptoms appear

If the original course lasting for three weeks is given, clinical improvement occurs steadily, the fever coming down after the 4th or 5th injection, the spleen decreasing in size and blood improving. In the intensive course, the clinical symptoms are not often influenced till the end of the course; the curative processes continue. The criterion of cure is the negative culture for leishmania.

Aromatic diamidines.

The Amomatic diamidines are toxic and should be used with care and preferably in antimony resistant cases. Stilbene is given in doses of 1 mgm per pound body weight; the adult dose is 0.025 gm as initial dose. If this gives a reaction next day the dose given is 0.035 gm, but it is proceeded by an injection of 0.25 ccm of 1 in 1,000 adrenaline solution. If the reaction is mild the next dose is 0.05 gm without adrenaline. The doses are increased rapidly by 0.01 gm or 0.02 gm according to reaction produced till a maximum dose of 0.001 to 0.01 gm per pound body weight is reached. Adrenaline is given for the next dose whenever there is a marked reaction. Ten injections are usually given, a total dose of 0.7 gm per kilo body weight and 1.0 gm for resistant cases. Toxic reactions are common.

Penicillin has been tried in Kala-azar, but has proved a complete failure.

The above courses—laid down are for Indian patients. In Sudan, China and other places a more intensive course is necessary.

CUTANEOUS LEISHMANIASIS

ORIENTAL SORE—MUCO-CUTANEOUS (SOUTH AMERICAN LEISHMANIASIS): LEISHMANIAL DYSENTERY.

1. Cutaneous Leishmaniasis or Oriental Sore

This is cutaneous infection with *Leishmania tropica* and is known by various names, *e.g.*, Lahore sore, Delhi boil, Baghdad sore, etc. The lesions (which may be single but are often multiple) occur on exposed parts of the body, infection being conveyed by the sandfly.

Epidimiology.

Oriental sore is widely distributed throughout the world, *i.e.*, Europe, North Africa, South America, Asia Minor, Syria, Palestine, Arabia, Persia, etc. In India it occurs in the dry portions of the Indo-Gangetic plain, the Punjab, North West Frontier Province, Baluchistan and West coast of the Peninsula. *P. argentipes,* the transmitter of kala-azar, breeds in moist areas and *P. papatassi* and *P. sergenti,* the transmitter of oriental sore is the sandfly of the dry regions. The disease is endemic in certain cases, but may assume an epidemic form; such an epidemic occurred on the outskirts of Delhi in 1939-40 when 15 to 20 thousand people were affected. In the North West Frontier Province and in the Punjab, cases begin to appear in June and July and the incidence is highest in August and September. In Quetta the maximum infection occurs in September and October. All races and ages are susceptible.

Oriental sore and Kala-azar both occur as a natural disease in dogs and the parasite found—*L. canis* is serologically the same as *L. donovani.*

Aetiology.

The mechanism of transmission is similar to that of kala-azar. *P. papatassi* and *P. sergenti* become infected by feeding on the indurated edges of the sore and ingest *L. tropica;* the development of the flagellate takes place in the gut and the organism moves forward towards its mouth; the infection is then conveyed to the new host by bite. Infection may

occur by direct inoculation from the sore but this is not the usual mode of transmission. A non-human reservoir of infection is suggested and lizards are suspected. In Aleppo and Tehran, dogs are heavily infected and probably act as reservoir.

Immunity. That there is some immunity is shown by the fact that in endemic areas, adults are not affected and only emigrants and children get the sore. After natural infection in man immunity is said to be lasting. Auto-inoculation is quite common so that immunity cannot be complete especially against hetrologous strains. Oriental sores do not confer immunity against other leishmania infections and vice versa.

Pathology. The presence of the parasite produces infiltration of the layers of the dermis by macrophage cells many of which contain parasites; this extends to the subcutaneous tissues; giant cells are sometimes present. The parasites get into the endothelial cells of the capilliaries which become swollen; the blood channels thus become blocked and necrosis results. Cellular proliferation interferes with the blood supply of the epidermis which is easily damaged by trauma. Pyogenic organism gain entry and ulceration occurs; the leishmania organisms are destroyed when this occurs and are difficult to find. The margins of the ulcer show thickening of the epidermis and finally the granuloma formed is invaded and superseded by fibrous tissue; when the septic process resolves the ulcer heals leaving a scar. Leishmania as a rule do not occur in the peripheral blood stream.

Symptomatology. The incubation period may vary from a few weeks to many months; in the majority of cases it is at least three months. There is a small itching red pappule surrounded by a pink area at the site of inoculation. This increases in size from 1 to 3 cm in diameter and a crust is formed with dry whitish or dark red scales. This breaks down in the centre forming an ulcer with clean-cut edges with a sloughing and later a granulating base, surrounded by an area of red induration. If no treatment is given, the sore usually heals after a year or so. In neglected cases ulceration extends in size and depth and becomes secondarily infected. Much disfigurement may result if the sore is on the face.

In some cases, a cauliflower-like growth may be formed, which may involve a large area. There are also non-ulcerating forms which may take the shape of a fleshy nodule or keloid or lupoid forms.

Diagnosis. The sore can be easily diagnosed by examination of the lesion for leishmania. In early lesions the organism is easily found, but in old ones, especially where there is infection with secondary organisms, it is often not possible to demonstrate leishmania. In such cases the sore is cleaned with alcohol and washed with saline; the spreading edge is then punctured so that it bleeds; the first blood is wiped with a sterile swab and a smear is made from the serous fluid which oozes or a culture may be made in N.N.N. medium.

The sore should be differentiated from tropical sore, Yaws, Veld sore, etc.

Treatment. In old days the treatment was mainly local, the ulcer was scraped with a Volkmann spoon under an anaesthetic till all friable tissue was removed from the edges and the base, and pure phenol was applied. The wound was then aseptically dressed and it healed up leaving a bad scar. This procedure is rather drastic and is not used now-a-days.

Local application of 2 to 4 per cent tartar emetic ointment has been suggested but is very painful; powdered potassium permangnate, mercurous or mercuric chloride, methylene blue ointment, powdered sulphonamide, carbon dioxide snow (applied for 5 to 30 seconds and repeated every other day), have been tried. When x-rays are available their application is said to cure the ulcer in 10 days.

Treatment by ionisation is believed to be effective. The ulcer is cleaned and then covered with a pad consisting of many layers of lint soaked in 2 per cent zinc solution. This is firmly applied with a bandage and then connected with positive pole of a battery and a current of 36 to 38 volts is passed. The application is continued for 20 minutes, the pad being kept moist. Diathermy has also been tried but not with much success.

Local injections of emetine hydrochloride 2 to 5 per cent solution have been given beneath and around the thickened edges and bases of the ulcers;

about 20 minims of the solution is injected at a time and several punctures being made. Mepacrine hydrochloride solution have also been used in the same way.

The best results have been obtained with berberine sulphate and Napier recommends the following technique:—

"If the ulcer is septic, hot magnesium sulphate fomentations and frequent dressings should be used for a few days to make the wound as clean as possible. A 2 per cent solution of berberine sulphate is used; this is injected by means of a tuberculin syringe into the indurated area surrounding the ulcer; about six injections will be required for each ulcer in order to infiltrate the whole circumference of the ulcer, but 1 ccm of solution will usually be sufficient for an average-sized ulcer. There will usually be some inflammatory reaction, which should be allowed to subside before further injections are given; it will usually be possible to give the injections once a week. From three to six treatments will effect a cure. If there are multiple sores, not more than two or at the most three should be treated at one 'sitting', but treatments can be given daily, the ulcers being taken in turn. This treatment, however, cannot be recommended when there are more than half a dozen ulcers."

General treatment, such as intravenous injections of tartar emetic in the same way as in the treatment of Kala-azar have been used when the ulcers are multiple. Pentavalent compounds (neostibosan), although they cured the ulcer, did not give such good results as trivalent compounds.

Fouadin starting with 1.5 ccm and increasing to 5 ccm intramuscularly on alternate days till 8 to 10 injections are given to produce healing.

Napier found aromatic diamidines useless.

Summary. According to Napier "in those cases in which there are single or only a few early sores and non-ulcerating lesions, beberine sulphate is recommended; in cases with numerous small or moderate-sized lesions, fouadin or neostibosan injections should be given; and in all cases with extensive ulcers heavily infected with pyogenic organisms, recourse should be had to surgical treatment. Until the sore is obviously healing sulphonamide powder should be included in all dry dressings. A judicious combination of surgical treatment with antimony injections will produce the best results in cases with very numerous extensive ulcers."

Under proper treatment the sore heals up in 2 to 3 weeks.

2. Muco-Cutaneous (South American Leishmaniasis)

This is also known as espundia and is a specific condition of the skin produced by *L. brazilensis* and transmitted by phlebotomus. The disease occurs in Mexico and many parts of South America.

Pathology. The parasite is readily found in the cutaneous lesions in the early stages but with difficulty later; they may be found in intact mucous membrane. There is no essential difference between cutaneous lesions in this condition and those which occur in oriental sore. In infection of the mucous membrane, which occurs in 10 to 20 per cent of cases, there is œdema of the submucosa and perivascular lymphatic infiltration, in which lymphocytes predominate; endothelial cells containing leishmania are present. In the second phase the mucosa is swollen and there is disquamation of the epithelium over the infected focus, and superficial necrosis covered by fibrinous exudate. Ulceration and necrosis in the nose, mouth and pharynx may result in extensive and distressing lesions which may lead to profound cachexia.

Clinical aspects.

In the primary phase, there is cutaneous ulceration, and in the secondary phase, there is infection of the mucosa of the buccal cavity and upper respiratory tract. The incubation period is generally 2 to 3 months. The lesions are ulcerating and non-ulcerating and the latter may be papillomatous or cauliflower-like or atrophic, looking like a red plaque with raised edges. The ulcerating type starts as an itching papule which goes on to pustule formation and eventually an ulcer is formed which may be 9 to 10 cm in diameter. The ulcers are round in shape, have undermined edges and a purulent exudate. The papillomatous type begins in the same way and also exudes serous fluid.

The mucosal lesions take 6 to 18 months to develop and the nasal lesions take much longer. The ulcers start as œdematous swellings which break down giving rise to small granular ulcers which may destroy the whole of the mucous membrane of the nose. They do not affect the tongue or the bones.

Diagnosis.

This is made easily in the early stages by pricking the edge of the ulcer and making a smear from the exudate. In case of mucous membrane, leishmania may be found by making a smear from the exudate obtained by scratching the intact surface. Montenegro's intradermal test is specific. One ccm of a suspension of a culture of *L. brazilensis* in 0.4 per cent phenol is injected intradermally. There is a sharp local reaction in 48 hours, which persists up to 72 hours. The test becomes positive after about a month and remains so throughout the course of the disease.

The disease has to be differentiated from Yaws, leprosy, syphilis and blastomycosis.

Prevention can only be carried out by protection from sandfly bites. A thorough treatment of cutaneous lesions will prevent serious mucosal lesions.

Treatment.

Local treatment is the same as in case of oriental sore. For mucosal lesions antimony has been the mainstay. Tartar emetic has been tried by the intravenous route and fouadin has been successful, the latter is given intramuscularly in 6.3 per cent isotonic solution in doses of 1.5 ccm increased to 5.0 ccm; injections are at first given daily and then on alternate days till 15 to 30 are given. Yatren given intravenously along with fouadin is said to accelerate healing of mucosal lesions. Arsphenamine compounds cure cutaneous lesions, but have no effect on the mucosal lesions. Atebrine injected locally and by the mouth is said to be specific for cutaneous lesions.

For mucosal lesions a gargle with sodium bicarbonate solution and wash with 0.1 per cent solution of tartar emetic is recommended. After anaesthetising with a 1.0 per cent cocaine solution with 1 per cent phenol, application of a 2 per cent tartar emetic is said to be effective.

3. Leishmanial Dysentery

In generalised infection with *L. Donovani* ulceration of small and large intestines is commonly seen as a terminal complication. In infantile kala-azar in Sicily entero-colitis with circular ulcers in the large intestine are a common occurance. The jejunum is thickened without ulceration and columnar epithelium of the villi disappears. L. D. bodies can be demonstrated in scanty numbers in the sub-mucous coat, and in the base of the villi. In the centre of the villi they multiply rapidly and are present in the endothelial cells in large numbers. Bodies resembling L. D. bodies are found in the stools of K. A. patients in whom dysentery-like symptoms have been induced with croton oil. These findings have been employed as an argument in favour of faecal transmission of kala-azar and infection has actually been produced in hamsters from stools of kala-azar patients.

CHAPTER IV

MALARIA

GENERAL CONSIDERATIONS: GEOGRAPHICAL DISTRIBUTION: MALARIA PROBLEM IN INDIA; AETIOLOGICAL CONSIDERATIONS: MALARIAL PARASITES AND VECTORS; EPIDEMIOLOGY: ENDEMIC INDICES; PATHOLOGY OF MALARIA; CLINICAL ASPECTS: ACUTE PYREXIAL ATTACK, SYMPTOMS IN DETAIL; DIAGNOSIS OF MALARIA; IMMUNITY IN MALARIA; MODE OF PRODUCTION—CHEMO-THERAPY OF MALARIA: STUDY OF ACTION OF ANTIMALARIAL DRUGS: TEST IN BIRD MALARIA, TEST IN MONKEY MALARIA, THERAPEUTIC TESTS; STUDY OF CURES: NATURAL CURE, ARTIFICIAL CURES, EFFECTIVENESS OF ARTIFICIAL CURES, COMPLETE CURES—THE CINCHONA DERIVATIVES: TOTAL ALKALOIDS, TOTAQUINA; ALKALOIDS OF CINCHONA; THERAPEUTIC EFFICACY; QUININE SUPPLY OF INDIA—PHARMACOLOGICAL ACTION OF CINCHONA ALKALOIDS: ACTION IN MAN—THERA-PEUTICS OF CINCHONA ALKALOIDS: QUININE IN MALARIA: SYNERGIST OF QUININE, DOSAGE, CAUSES OF FAILURE OF QUININE THERAPY, RELAPSES; ROUTES OF ADMINISTRATION; OTHER CINCHONA ALKALOIDS IN MALARIA; QUININE IN OTHER CONDITIONS—TOXIC EFFECTS: QUININE IDIO-SYNCRACY; CINCHONISM; VISCERAL DEGENERATION; TREATMENT OF CINCHONISM—MODE OF ACTION OF QUININE: SUMMARY OF TREATMENT OF MALARIA WITH CINCHONA ALKALOIDS—PREPARATIONS OF CINCHONA ALKALOIDS & ITS DERIVATIVES—PLASMOQUINE: PHARMACOLOGICAL ACTION; EFFECTIVENESS AGAINST MALARIA—MAPACRINE (ATEBRIN): PHARMACOLOGY; EFFEC-TIVNESS AGAINST MALARIA; TOXIC EFFECTS—OTHER DRUGS USED IN MALARIA—THE NEW ANTIMALARIAL DRUGS: PALUDRINE: EXPERIMENNTAL WORK, BIOLOGICAL RESULTS, PHARMACOLOGY & TOXICITY, PALUDRINE IN HUMAN MALARIA; CHLOROQUINE (S. N. 7618); METACHLORIDINE (S. N. 11,437); PENTAQUINE; SUMMARY OF TREATMENT OF MALARIA WITH PALUDRINE—SUMMARY OF TREATMENT OF CLINICAL MALARIA—PROPHYLAXIS OF MALARIA: GENERAL MEASURES; CHEMO-THERAPEUTIC SUPPRESSION; PREVENTION AND CONTROL OF MALARIA IN WORLD WAR II—SPECIAL FORMS OF MALARIA: CHRONIC MALARIA; MALARIA IN CHILDREN—BLACK-WATER FEVER: GENERAL; TREATMENT—INDUCED MALARIA: GENERAL; TREATMENT—ANTIMALARIAL DRUGS IN SIMIAN MALARIA—PRESENT POSITION OF ANTIMALARIAL DRUG THERAPY: CINCHONA ALKALOIDS; SYNTHETIC ANTIMALARIAL DRUGS: THE AMINO-QUINOLINE DERIVATIVES (PLASMO-CHIN, PENTAQUINE, CHLOROQUINE), ATEBRINE AND SIMILAR COMPOUNDS, SULPHONAMIDES, THE I. C. I. SERIES (PALUDRINE); CINCHONA ALKALOIDS VERSUS SYNTHETIC ANTI-MALARIAL DRUGS.

1. General Considerations

Introductory.

The periodic attacks of fever coming on with shivering and passing off with sweating have been recognised as a clinical entity for many hundred years. In the *Charaks Samhita* there are references to fevers spread by mosquitoes and it has been suggested that these refer to malarial fevers. Such fevers were also known at the time of Homer. The term malaria, however, was employed for the first time for this disease by Italians under the impression that the fever was due to miasmatic exhalations from marshy grounds (mal-bad, aria-air). Many other names have been given to this disease from time to time; some of these names, such as marsh fever, tropical fever, coastal fever and acclimatization fever, were indicative of the supposed origin of the disease, while others, such as ague, and febris intermittens had their origin in the chief characters of the disease. Hippocrates definitely established the entity of the disease and further divided the intermittent fevers into three main groups quotidian, tertian and quartan.

Synonyms.

Definition.

Malaria by present day usage is not defined on a clinical but on a parasito-logical basis, *i.e.*, the term means infection by one of the malarial parasites (Christrophers). It is characterized by fever, anæmia, enlargement of spleen and sometime fatal complications.

The Malarial Parasites.

The malarial parasites belong to the class *Sporozoa,* Sub-class *Coccidiomorpha,* order *Haemosporidiidea,* and genus *Plasmodium.* A number of species of Plasmodia are recognized, the better known ones being *P. vivax, P. falciparum,* P. *malariae* and *P. ovale* infecting man; *P. knowlesi* and *P. inui* infecting monkeys, and *P. relictum, P. gallinaceum* and *P. lophurae,* infecting sparrows, chicks and

ducks respectively. Plamodia are amœboid organisms which inhabit the tissues and erythrocytes of the vertebrate hosts (man, monkeys, birds, cattle, etc.), and produce a characteristic pigment. They have an asexual cycle or schizogony, and a sexual cycle or sporogony, which occurs in the erythrocytes of the vertebrate host. In P. gallinaceum of chicks, the schizogony cycle has also been shown to occur in the cells of the reticulo-endothelial system, but sporogony (sexual cycle) occurs in the red blood corpuscles only.

Life cycle.

The asexual cycle occurs in the erythrocytes, and, at least in some species, it has also been shown to occur in the cells of the reticulo-endothelial system. Exoerythrocytic or tissue parasites have recently been demonstrated in mammals, this stage is not pigmented since pigment is derived from heamoglobin of red cells. It is possible that reinvasion of the reticulo-endothelial system may occur from the blood. When sufficient numbers of the asexual parasites are present in the blood, febrile symptoms appear in the host. These asexual froms are incapable of infecting mosquitoes but will cause an infection if the infected blood is injected into another person. Gametocytes are also formed in the red blood cells, but these alone can produce no symptoms. When a female anopheline mosquito sucks blood containing such gametocytes, these latter undergo development in the insect's body with the eventual production of large numbers of sporozoites (sporogony cycle), which find their way to the insect's salivary glands, and the mosquito then becomes infective to man.

Importance of Malaria.

It has been rightly said that malaria is one of the greatest scourges of humanity. It is a menace to any people or country in which it has a decided incidence In the number of deaths caused either directly or indirectly, the sickness and suffering, the loss of time and efficiency, the expense, the lowered vitality of the afflicted and the reduction of the value of property, malaria is without rival among the diseases afflicting mankind (Leathers, 1918). During the first Great War it caused the greatest loss of effective man-power, the largest invaliding and subsequent pension roll of any one disease. In 1932 the League of Nations Malaria Commission sent questionnaires all over the world to more than one hundred governments of countries infected with malaria. From replies received, it was apparant that approximately 17½ million cases of malaria had been treated. In India alone only 8 to 10 million of a possible 100 million cases were considered as being treated. The total number of cases all over the world must, therefore, be enormous. A resume of its geographical distribution was given by Rogers (1928) who showed how widespread the disease is all over the world.

(1) Geographical Distribution

General.

Malaria reigns over a vast domain extending from latitudes 60°N up to 30° to 40°S. It is mainly a tropical disease but is not limited to tropics, being prevalent in sub-tropical and temperate countries as well. The distribution of different species of malaria is closely related to the summer mean isotherms. The distribution of malaria in general throughout the world is limited in northern and southern hemispheres by the summer mean isotherms of 60°F; the distribution of *P. falciparun* by the mean summer isotherms of 70°F; the distribution of *P. malariae* shows a most curious and patchy distribution, suggesting that this is a dying species. *P. ovale* and *P. tenue* have a restricted distribution; the former having been recorded in the East as well as the West (Tropical, Africa and also in South America), while the latter has been found among aboriginal tribes in some parts of India.

The detailed distribution of malaria in India is given below:—

Malaria in India.

The distribution of malaria in India shows great diversity, because the climatic features of India are very diverse. The valley of Kashmir at about 4,500 feet above sea level is strangly free from malaria, but malaria exists in the Himalayas at Murree at—an altitude of over, 8,000 feet.

Regional Distribution.

The Indus plain, from the North Western Frontiar to Delhi, shows negligible quartan malaria with benign tertian infection predominating in the spring and malignant tertian in autumn and early winter. It is the region of vast periodical epidemics caused by the latter named parasite. The Gangetic plain from Delhi to Calcutta is an area of moderate

to high endemicity. In the United Provinces, benign tertian is the predominating variety and in Behar benign tertian and malignant tertian infectious occur in about equal proportion; the incidence of the quartan parasite is low. Hyperendemic area occurs in the Nepal Terais at the foot of the Himalayas. In Bengal, there are hyperendemic areas in the western districts and in some scattered areas in North Bengal, the Dooars, Darjeeling Terais and Chittagong hill tracts. In these areas *P. falciparum* is the predominant variety. (In Bengal incidence is:—*P. falciparum* 46.3 per cent, *P. vivax* 41.4 per cent and *P. malaria* 12.3 per cent). In North Eastern India, areas of hyperendemicity occur in the Assam valley and at the foot of the Himalayas. The rest of the area shows moderate endemicity with liability to autumnal increase.

South of the Indo-Gangetic plain in the belt of high land which forms the northern escarpment of peninsular India, the Vindhya, the Arballi and Chota Nagpur, there is a tract of extreme malarial prevalence especially towards the east. In Madras Presidency along the Eastern Ghats, the region shows moderate endemicity with areas of hyperendimicity, and a hyperendemic zone stretches along the Western Ghats to as far south as Travancore State. The rest of the Deccan is an area of moderate endemicity.

Distribution of the Parasite

Seasonal Variation.

Regarding the distribution of the varieties of the parasite, *P. vivax* is the predominant species, but this only applies to the spring and early summer months. In autumn and early winter *P. falciparum* usually becomes predominant. This applies to all India generally.

The distribution of *P. malariae* in India is an interesting study; it is minimal in northwest India. The importance of *P. malariae* becomes increasingly greater as one passes from the North West Frontier Province to the east and south west. The fact that *Plasmodium malariae* is least prevalent in north west India (which is separated from the malarious parts to the west by the Pamirs and the Persian desert) and that its incidence gradually increases eastward, tends to show that this variety entered India from the east.

Other Countries.

Malaria occurs in Afghanistan, Persia, Mesopotamia, Arabia, Asia Minor, Turkestan, over a wide zone extending over Central Asia, Burma, Siam, China, Formosa, Japan, and the Phillipines. Certain islands in the Pacific like Hawai and Fiji which are widely separated from the mainland are free from malaria.

Malaria in Europe.

Malaria existed in the lowlying parts of England, and it occurred in Finland and the low lands of Germany, in Denmark, South Sweden and Scotland. Vast areas in Italy had become depopulated and were abandoned because of the heavy incidence of malaria. Vigorous anti-malarial measures have since been adopted and flourishing towns are now arising in areas like the Pontine marshes. Greece and Southern Balkans, were heavily infected even as late as 1914-18 but the disease is now being successfully combated. Malaria occurs in Poland, Hungary and Southern and South Western Russia, In Europe the carrier of malaria is *Anopheles maculipennis,* a homogeneous species with at least six widespread, varieties, differing more in biological than in physical traits.

Malaria has shown a striking recession in Europe during the last half century. This recession has been ascribed to various factors. It is apparently not due so much to reduction in the numbers of the carrier mosquito as to social and economic improvements and in many areas and to the stall feeding of cattle leading to deviation. Vigorous anti-malarial measures by the respective governments are also greatly responsible for the decline of malaria in these countres.

Malaria in Africa.

Malaria is widespread in Africa. The whole of the equatorial region down to the northern part of the Union of South Africa and the northern coast down to the desert are affected. Subtertian malaria is the commonest form in the cases of Northern Africa and East and West Africa. *P. ovale* occurs mainly in Nigeria, Congo and Uganda.

Malaria in America.

Malaria occurs in the southern and middle States, in the Panama Canal zone, and the West Indies but is generally on the decline. Malaria is endemic on the east coast of South America, South of Riode Janero and inland along the great rivers and their tributaries; benign tertian malaria occurs in the Andes at an altitude of 9,000 feet and extends to the southern limits of Argentina.

Malaria in Australia.

In Australia malaria occurs on the north coast and in a mild form; the whole of New Guinea is infected. Quartan malaria is fairly common in the South Seas and adjacent islands.

Malaria Problem in India

Intensity and virulence.

It is true that in India there are few malarial foci where the disease attains the same degree of virulence and intensity as it does, for example, in Africa, Southern Italy and Greece, but the disease is so prevalent and widespread that India, taken as a whole, probably is one of the most malarious countries in the world and the disease constitutes a major public health problem, both from the point of view of morbidity and mortality. The Annual Report of the Public Health Commissioner with the Government of India, shows that during 1936, malaria accounted for about 1,567,000 deaths. During the last decade cholera, small pox and plague, the three infectious diseases which generally attract most notice were together responsible for about 357,000 deaths each year—a figure which is less than one-third of the annual toll of life taken by malaria.

Mortality and Morbidity.

National Suffering.

Regarding morbidity, it has been estimated that near about 100,000,000 persons suffer from malaria every year. Besides causing an appalling mortality and morbidity, malaria leads to retardation of national development in India. It reduces normal birth rate and is the greatest factor in lowering the health, vitality and physical development of the people. This has the deplorable effect of hindering greatly the intellectual development of the affected people. The financial loss due to direct and indirect effects of malaria is enormous. The financial loss to the individual and the family alone has been calculated at not less than 11,000 lakhs of rupees per annum. The financial and economic losses which the government and administrations have to bear through the ravages of this disease are enormous. The disease produces not only a decrease of revenue but also an increase in the cost of administration. Malaria causes incalculable loss to agriculture, industry and commerce, mainly through its direct and indirect action upon the labour population.

Economic Loss.

Fortunately this is one of the diseases against which true specific remedies are available.

(2) Aetiological Considerations

Historical.

Long before the cause of malaria was discovered the disease could be more or less successfully treated by cinchona alkaloids and Binz from his experiments with quinine and protozoa prophesied the discovery of an organism belonging to this order as being the cause of malarial fever. The prophecy was fulfilled in 1880 when Leveran discovered the plasmodium in the blood of patients suffering from malaria. Leveran's findings were corroborated soon after. The different stages of development of the quartan parasite in the blood were demonstrated by Golgi in 1885, and in the following year that of the benign tertian parasite, as well as its connection with the course of fever.

Regarding the transmission of the disease, there was a popular belief in several parts of the world that mosquitoes were responsible. King in 1882 gave a number of reasons for believing that malaria was transmitted by mosquitoes and in 1894 Manson formally suggested that mosquitoes might be the vectors of malaria.

MacCallum (1897) observed the fertilization of the halteridium parasite in birds and the fertilisation of the macrogametes. Ronald Ross in 1897 described the development of the pigmented bodies, now known as oocysts, in the stomach wall of mosquitoes made to feed on malarial patients and later described the complete development of bird malaria in the mosquito. In 1898 Grassi described the complete cycle of development of the malignant tertian parasite in anopheles, and also that of other forms. In 1900 Manson experimenting in England, a malaria free country, was the first to transmit malaria through bites of experimentally infected mosquitoes.

Malarial parasite and vectors

Human species.

The malarial parasite is a protozoon belonging to the genus plasmodium. The human malarial parasites are not capable of thriving in the blood of any of lower animals except some of the higher apes; man is thus the only reservoir of infection of human malaria. The existence of three species, *i.e., P. vivax* (benign tertian), *P. falciparum* (malignant tertian) and *P. malariae* (quartan) is confirmed and recently *P. ovale* has also been described. There is a tendency to further subdivide these recognised species into minor varieties, and the plurality view has been recently strengthened by thousands of inoculations carried out in the treatment of general paralysis of the insane and other nervous diseases. Cultures of the parasites have furnished additional evidence. Stephens has described *P. tenue,* and Marchouz in 1925 suggested that each of the three recognised species of malarial parasites exhibits different races and morphology with varying geographical distribution.

Simian and Avian Malaria. A malarial parasite of monkeys was first reported by Koch (1898). Since then several workers have noted the occurrence of plasmodia in different species of monkeys. *P. Knowlesi* was found in *Silenus irus* monkeys in Calcutta. When inoculated into a *Rhesus monkey* (common Indian species) this parasite produces a very intense and virulent infection which usually terminates fatally if proper treatment is not given. For this reason Rhesus monkeys infected with *P. Knowlesi* are very well adapted for testing the therapeutic efficiency of anti-malarial drugs. This monkey plasmodium has been successfully inoculated into human beings. In man, one attack of monkey malaria confers absolute immunity against further inoculation with the same parasite. *Simian species.*

The malarial parasite of birds are of particular interest in connection with the investigations of Ross who traced them through all the stages of their sexual cycle in the culicine mosquito. *Avian Species.*

Several species of bird plasmodia have been described. The commoner variety, *P. marcox* has worldwide distribution; sparrows being the type host of this parasite. In some species, such as *P. gallinaceum,* the parasite in domestic fowl, in addition to the schizogony in the red blood corpuscles, schizogony also occurs in the cells of the reticulo-endothelial system. Whether exo-erythrocytic schizogony also occurs in human malaria, has not yet been determined.

Small mammals, such as bats, flying foxes and squirrels often show plasmodial infection. With regard to large mammals, plasmodia have been found in the Indian buffaloes. Plasmodial parasites have also been described in the blood of lizards. *Other species.*

Vectors of Malaria. The following points of practical importance with regard to the vector of malaria must be noted.

1. Only some of the species of anopheline mosquitoes can transmit malaria.
2. Only the female mosquito acts as a transmitter.
3. In any locality only one or two species of the anopheline mosquitoes are responsible for the transmission of malaria.

This last factor is important from the point of view of prevention. Knowledge of the breeding habits of the mosquitoes responsible in a locality and the adoption of such measures as would adversely affect breeding conditions, may secure effective control over the disease in the locality. Thus in India, out of 43 existing species, only 12 are capable of transmitting malaria and following 9 are important:—*Anopheles minimus, fluviatlis, varuna, culicifacies, sundaicus, stephensi, phillipinensis, annularis* and *pallidus.* *Indian mosquitoes.*

Duration of infection in mosquitoes. The question as to how long anophelines once infected with malaria can remain infective to man has also been worked out by inoculation experiments. Infection could be produced by bites of *A. punctipennis* up to 55 days after feeding on crescents, and sporozoites were found in it.

(3) Epidemiology

There are three essential links in the aetiological cycle of malaria:—(1) Man (the reservoir of infection), (2) malarial parasites (the causative parasite), and (3) the mosquito (the transmitting agent). There are certain additional factors which are essential for the successful transmission of malaria, important amongst these factors being the amounts of rain-fall, the temperature and humidity, and the immunity of population, which is in turn affected by famines, movement of the population and other economic conditions. *Aetiological cycle.* *Factors affecting Endemicity.*

Malaria is mainly a disease of damp low lying areas, such as foot of hills, river banks, and sea coasts. On the other hand it may occur even in deserts and big cities which appear to be ordinarily immune. In deserts, small collections of water, such as lakes and springs, provide breeding places for the mosquito, while in big cities, the mosquitoes find breeding places in underground cisterns and in house tanks and reservoirs (as in Bombay and Calcutta). Temperature of about 60°F is necessary to enable the parasites to develop and malaria to be prevalent. This mean temperature must be maintained for sixteen days to allow full development of *P. vivax,* while in Macedonia a higher temperature is necessary for development of *P. falciparum.* Elevation and the consequent low temperatures limit the prevalence of the disease. In Kashmir at an elevation of 6,000 to 8,900 feet, *A. maculipennis, A. fuliginosis* and *A. wallmori* are present in abundance, but no malaria exists because the temperature is not high enough for the parasites to develop. Thus, altitude *Temperature.* *Altitude.*

Epidemics.

is also important although indirectly. Malaria, however, may be present in the valleys of hills where the hill streams form a favourable breeding place for certain species of Anopheline mosquito. In the Punjab, where epidemics occur after every six or seven years, the main factors according to Gill (1923) are:—The high relative humidity resulting from heavy rainfall in July and August. (2) A low spleen rate, indicating absence of temporary immunity due to recent attacks of malaria. (3) Economic distress and high price of food. (4) The occurrence in previous years of outbreaks of malaria.

Endemic Indices

Endemic Index.

In estimating the prevalence of malaria in a locality determination of "splenic index" is helpful. The term denotes the percentage of children between the ages of 2 and 10 years who have enlarged spleens due to malaria. According to Stephens and Christopher the age limitation is important, for among the inhabitants of a very malarious country the adults are more or less immune and their spleens are diminished in proportion. The infantile spleen rate *per cent,* is the basis of endemic malariousness of a locality.

Splenic Index.

Endemic Index.

Determination of the parasite rate by the thick film method, is of greater value in estimating the prevalence of malaria than the spleen index. When twenty-five or more slides of unselected village children under 10 years of age are taken and examined the percentage in which malaria parasites are found is termed the *endemic index* of the community. Besides the spleen and parasite rates, an endeavour should be made to ascertain the amount and severity of "incidental malaria" among the immigrant population, and the percentage of infection in anopheline mosquitoes (the sporozoites rate).

Methods.

A number of methods of determining the degree of splenic enlargement *e. g.* that of Christophers, Schuffners, etc. have been described.

The most convenient method followed in the School of Tropical Medicine, Calcutta, is to push the spleen as far as possible and measure the farthest point in inches from the tip of the ninth costal cartilage.

Technique of Endemic Indices.

It is necessary, before undertaking a malaria campaign, to estimate the amount of malaria present in the locality. This is done by determining the following indices:

(*i*) *The splenic index;* this is the percentage of children under 15 with enlarged spleens palpable below the costal margin.

(*ii*) *The splenometric index;* the projection of each spleen below the costal margin is measured in finger breadths and the average size of enlargement is determined. It is particularly useful in assessing the value of antimalaria measures in places where the splenic index is high and the spleens are large. Here the splenic index alone would not detect an improvement indicated only by a general decrease in size of the enlarged spleens, if they remain palpable. (*iii*) *The Parasite index,* is the percentage of children under 15 in whose blood parasites are found during a single examination of a thick drop preparation for a fixed period of 5 to 10 minutes. (*iv*) *The Endemic Index of Ross.* This is obtained as follows; the number of children with parasites in their blood and the number of children with enlarged spleens but no parasites, is divided by total number of children examined. This index is useful in the comparison of different localities. (*v*) *The sporozoite indices.* This is the percentage of anopheles with sporozoites in their salivary glands. The following technique is recommended:—remove the legs and wings of the mosquito; fix the thorax by transfixion with a pin; seize the head with fine forceps and pull it off; two shining drops are visible at the back of the head, these are the salivary glands; spread them out on a slide; sometimes the glands remain in the thorax.

(4) Pathology of Malaria

Blood

Red blood corpuscles.

The malarial parasite, is a true blood parasite, living in the red cells. With each attack of pyrexia, the loss of red cells may be a million or more per cubic millimeter of blood, within 24 hours. The destruction due to actual invasion of parasites is greater in subtertian than in benign tertian and quartan infections; it is greatest during the first few paroxysms and becomes gradually less in later attacks. In the malignant type there is evidence of toxic destruction of erythrocytes. Although during the apyretic intervals the red corpuscles increase rapidly, when the disease has lasted for sometime anaemia is present; in some chronic cases it becomes very marked, and the changes may resemble those in pernicious anaemia.

Signs of a secondary anaemia of any degree may appear. Reticulocytes which are the immature erythrocytes appear, and there is a tendency of the parasites to attack these cells, particularly of *P. vivax*. Megalocytes are rare, but basophilic granular erythrocytes are very characteristic of chronic malarial anaemia.

Disintegrated red blood corpuscles are frequently seen in blood preparations in malaria, some being present in the circulating blood and some are produced in the course of smearing. Giant forms first described by the brothers Sargent are seen.

An increased resistance of erythrocytes to saline solution in malaria infected persons, especially more marked in malignant tertian and in chronic cases with enlargement of the spleen and liver has been observed. The sedimentation rate of red blood corpuscles may be increased during the acute phase of the infection, but becomes normal again with treatment.

Blood platelets.

During attacks the number of blood platelets is reduced, later on, in the stage of leucopenia, it is often increased.

Haemoglobin.

Reduction of haemoglobin is due to reduction in red blood corpuscles and the low haemoglobin content of the cells. It seldom returns to the normal standard so quickly as the red blood corpuscles.

White blood corpuscles.

Changes in the white blood corpuscles and red blood corpuscles are useful chiefly for diagnosing malaria infections in chronic forms and when fever is suppressed by taking a few doses of quinine.

A true leucocytosis occurs during the first few paroxysms, especially in malignant tertian infection; but after several attacks leucopenia is characteristic of malarial fevers, the number being generally between 3,000 and 5,000 per cubic millimeter of blood. During the initial leucocytosis there is increase of the polymorphonuclear leucocytes, replaced later by mononuclear increase alone, with the appearance of Turck cells and Reider cells. Eosinophils decrease or disappear during the attack and increase in the interval even to the extent of producing a mild eosinophilia during convalescence.

Phagocytosis.

Phagocytosis by the white blood corpuscles is common in malaria. It is particularly seen in the large mononuclear and transitional leucocytes, and to a less degree in the polymorphonuclears. Mainly, portions of pigment are phagocytosed, rarely, especially in malignant tertian a phagocyte may engulf a red cell and its parasite or the parasite alone.

Certain cells, *e.g.* macrophages, vascular endothelium etc., which are absent from or are very rare in the blood of healthy subjects, enter into the peripheral blood.

Pigment.

The true malarial pigment melanin or haemozoin is a black pigment formed from hæmoglobin by the malarial parasites while living in the erythrocytes. When the parasites escape the pigment is taken up by phagocytes and destributed in the walls of the blood vessels, and blood forming organs; this causes a slate grey to black discoloration of the organs specially brain, spleen, liver and adipose tissue.

Hæmosiderin, a yellow pigment associated with blood destruction, also appears in malaria. It is found as granules, specially in liver cells, Kupffer's stellate cells, capillary endothelia, spleen, marrow, kidney, pancreas, and occasionally in the white blood corpuscles.

Spleen.

During each attack of malaria the spleen is moderately enlarged, sometimes painful and tender, but soft. In an untreated case its edge can be felt on palpation a few days later.

Early enlargement is an acute congestion due to accumulation of parasites and lowered vascular tone. The pulp looks black and the sinuses and trabeculae are congested. There is great proliferation of the reticuloendothelial cells which become highly phagocytic. The endothelial cells lining the sinuses are filled with iron containing pigment. The reticular cells of the pulp show similar changes.

In chronic malaria the spleen becomes very large and hard, weighing as much as 2 to 3 lbs or more, and may extend to the umbilicus or lower. It presents a slate grey to dark red or chocolate-brown colour. There is marked hyperplasia of the tissue element, a great thickening of the reticular fibres, and of the trabuculae of the capsule. Sometimes perisplenitis causes tenderness in the splenic area in early cases and later thickening of the capsule and adhesions to the neighbouring organs.

Liver.

In acute malaria the liver is frequently slightly enlarged and tender; a distended gall-bladder and jaundice may be present. In chronic cases it may be very large, hardened and from brown to dark grey in colour. Cloudy swelling of the parenchyma cells or fatty

degeneration of the central areas of the liver occurs. Capillaries are distended with parasitized erythrocytes, macrophage and phagocytic endothelial cells. The jaundice which is not infrequent may be of toxic or hæmolytic origin.

Brain, Bone-marrow and other organs.

In the brain parasite-infected red cells, especially those with mature schizonts, have a tendency to cling to the sides of the blood vessels and gradual packing of the capillaries with these red cells and accumulation of pigment results in reduction of circulation, thombosis, blocking of the capillaries and symptoms of malarial coma.

The bone-marrow.

The bone-marrow is dark red in acute malaria. In chronic malaria, the marrow of the bones changes from yellowish brown to chocolate brown in colour due to the replacement of the fat by vascular tissue. Parasites are often found in the bone marrow.

The Kidney.

Generally the kidney shows little or no change, though acute hæmorrhagic type of nephritis is one of the rare complications which is usually sudden in onset.

Supra-renals.

Changes in supra-renals are more frequent in severe malignant tertian infection. The lesion generally is a hæmorrhagic necrotic inflammation of the capsule.

Pancreas.

There may be massing of parasites in the capillaries of the pancreas with small hæmorrhages and deposit of malarial pigment in the pancreatic tissue.

Digestive organs.

The digestive organs do not usually show any special changes. In case of localisation of infection, local disturbances may occur and gastric secretion is generally deficient. In the intestines capillaries of the mucosa and villi sometimes get blocked with parasitised erythrocytes, leucocytes and phagocytic cells containing pigment, producing small hæmorrhages and patches of necrosed epithelium.

Emboli caused by parasites may be found in the heart and lungs. The malarial pigmentation of the lungs results from migration of pigment-laden macrophages.

Malarial parasites and the malarial paroxysm

Febrile threshuld paroxysm.

The acute paroxysms of malarial fever are associated with the asexual development of the malarial parasites in the blood. Enumerative studies of parasites have been carried out on untreated cases of malaria due to different species of parasite, watched from day to day throughout their illness by Ross and Thomson (1910). These authors define a 'febrile threshold' for malaria; if the intensity of the infection rises above that threshold, the patient gets fever; if it is below the threshold, the patient—although infected—is afebrile (subclinical malaria). The actual numerical value of the threshold probably varies in different individuals. With *P. vivax,* about 200 to 500 parasites (asexual forms) per cmm of blood appear necessary to produce fever; with *P. falciparum* the corresponding figure is about 600 to 1,500 parasites per cmm with *P. malariae* slight rigor is present with counts of about 140 per cmm. There appears to be a correlation between the number of asexual plasmodia found in the peripheral blood and the fever. Nocht and Meyer, however, consider that it is improbable that the fever is just a direct consequence of malarial parasite increase. Whether it is a toxin liberated by malarial parasites, or the products of disintegration of parasitised red cells, or the malarial pigment which is liberated into the circulation during the asexual cycle, has not been established. The consensus of opinion is that the mechanism is similar to that of the fever associated with injection of foreign proteins.

The three stages of malarial fever, *viz.* shivering, fever, and sweating stage, can be correlated with three distinct phases in the development of the parasites. During the onset of fever and during the shivering stage, schizonts and young trophozoites predominate in the blood.

In malignant tertian, only in very severe cases, the schizonts are seen in the peripheral blood as the asexual division of this parasite occurs almost entirely in the internal organs. At the height of fever, the schizonts are least numerous, and the very young trophozoite forms are common. During the sweating stage progressive development of the parasites occurs, and this is completed in the apyrexial period. Peripheral blood shows maturer forms of the parasite, the trophozoites and schizonts in various stages of development, just before and at the onset of the next pyrexial attack.

(5) Clinical Aspects

Incubation period.

The incubation period in infection with plasmodium malariae (quartan) is 14 to 21 days, in infections with *P. vivax* (Benign tertian) 14 to 18 days, in infections with P.falciparum (malignant tertian) 8 to 12 days. These data are based mostly on observations in experimental production of malaria, by inoculation of infected blood, or by bites of infected

mosquitoes in human beings. The incubation period, however varies within wide limits, and the primary attack may be delayed for weeks or months (latent infections). The factors influencing the incubation period are related both to the host as well as the parasite. In the case of the host excessive physical strain, mental worry, exposure to chill, parturition and similar conditions that lower the general body resistance, help in shortening the incubation period and precipitating the attack. In the case of the parasite the number of sporozoites infected, and the number of merozoites produced by a particular variety *i. e.*, its rate of multiplication, are the determining factors. Acceleration or retardation of cycle may occur.

(i) Acute pyrexial attack

Prodromal symptoms.

The prodromal symptoms may be noticed a few days before the occurrence of the actual malarial paroxysm or just a short time before it. These are sense of fatigue, malaise, dull headache, pain in the extremities, pain over the spleen area, epigastric discomfort, anorexia and sometimes constipation. Some or all of these symptoms may be present in a particular case. Occasionally initial fever may be the only sign, as is the case with inoculation malaria. The prodromal symptoms may not be noticed and sudden onset of a chill may be the first warning of the attack. Those who have had frequent attacks of malaria easily recognise these symptoms and know that a paroxysm will follow.

Paroxysm.

Though the fevers produced by four different varieties of malarial parasites vary to some extent in their character, there are certain features common to all of them. The fever generally comes during the forenoon, (more correctly between midnight and mid-day) and this is a characteristic feature of diagnostic importance. The patient is usually aware that he is going to have a chill in a few hours before its advent. On an average the attack lasts from six to ten hours, the cold stage occupying about an hour, hot stage about four to six hours and the sweating stage from two to four hours (B. T. and Quartan).

Cold stage.

The cold stage follows on the premonitary symptoms. A chilly sensation spreads from the vertebral column to the limbs and jaw; shivering next supervenes shaking the patient violently with chattering of the teeth. The feeling of cold is so intense that the patient lies huddled up, covering himself with all the available wraps. The face has a pale, anxious look, the lips and nails appear cyanosed, and the skin exhibits the condition known as goose-skin. Respiration is shallow and quick, and the pulse is rapid and small. Nausea, bilious vomiting and severe headache are common. The skin temperature is low, but the internal temperature begins to rise, and is the highest by the end of this stage (about 104°F).

Hot stage.

The hot stage usually starts with a feeling of warmth and some relief from chill and shivering, but soon the patient feels intensely hot. The rectal temperature does not go beyond 103°F or so. The headache continues, the cheeks and the entire skin is flushed, and the eyes are congested. The pulse is full, bounding and rarely dicrotic. There may be pain in the splenic area. Within 4 to 6 hours the temperature begins to fall rapidly and the sweating sets in. Due to intense thirst the patient likes to take plenty of cold water.

Sweating stage.

The dry and hot skin become cool and moist. Perspiration first breaks out on the forehead and gradually the whole body is bathed in a copious sweat which has a peculiar spermlike smell. The temperature becomes normal or subnormal and the pulse slower, and of low tension. The patient feels comfortable and falls into a quiet sleep that may last for several hours.

The paroxysm of fever leaves the patient somewhat exhausted and weak, but those who frequently get malarial attacks usually go about their work after the paroxysm is over.

Course.

After a few paroxysms, or after the disease has persisted for ten days or more, the patient may get well without any special treatment. Relapses occur in the majority of cases; no method of preventing them has been discovered. Like other protozoal diseases, malaria is also a chronic disease, but if there is no further infection it definitely cures itself after some years. Spontaneous cure is relatively more rapid in subtertian (if the patient survives the acute attack) than in benign tertian and quartan malaria. In the former spontaneous cure takes place in one to two years, in benign tertian in four or five years and in quartan possibly a little longer. Malaria parasites do not remain in the blood beyond five years after continuous stay in a non-malarious place. During these years the infection persists, the patient may suffer from attacks of malaria as a result of anything that might lower his resistance, *viz.*, an accident, shock, exposure to inclemencies of weather, surgical operations, etc.

B. T. Infection.

Benign tertian or *P. vivax* infection is the common infection in the tropics and is the most predominant throughout the year in India. The shivering is less marked, the temperature rises very quickly, is more prolonged and falls rapidly. Headache, vomiting and pains in the limbs are worse than in quartan. The onset of paroxysm is more synchronous with the segmentation of plasmodia in this type which lasts for 6 to 10 hours and occurs every third day. In the case of double infection, fever may come on daily, and during epidemics several parasites of different ages may cause an irregular, more or less continuous fever. Hyperpyrexia and coma are the pernicious symptoms which rarely occur during the febrile stage, herpes of the lips or penis or urticarial or erythematous rash of the skin may appear. In a non-endemic area, 80 per cent of the relapses are due to benign tertian and 20 per cent to malignant tertian malaria.

Quartan infection.

P. malariae infection or quartan fever is generally the least common of the three types. In the tropics it appears after the rainy season is over. The paroxysms appear every 72 hours *i.e.*, every fourth day from which the fever derives its name (quartan fever). Double or triple infections have been reported, in which paroxysm occur on two successive days, or daily paroxysms may occur. Similarly mixed infections with different species, due to sporulation at various intervals, will result in an irregular or continuous fever.

Clinically the premonitory symptoms are less pronounced, the attack is more sudden and severe and the pernicious attacks are more frequent. Nervous symptoms and slight delirium are more marked during the febrile stage. Abnormally high temperatures are reported but a sub-normal temperature persist for a longer time during convalescence. The paroxysm is not so prolonged as in the benign tertian types and occurs most frequently during the day i.e., late morning or the early afternoon. On the whole the infection is more severe than bening tertian and has a higher mortality rate particularly in children.

M. T. Infection.

The incidence of *Malignant tertian* or *P.falciparum* infection is much higher in the tropical and subtropical regions than in the temperate zones. Cases may occur throughout the year, but the highest incidence begins in the later part of the rainy season.

The symptoms are more apt to be atypical and periodicity is difficult to observe. The fever is of the tertian type but each attack lasts 16 to 18 hours, sometimes as much as 40 hours, so that the apyrexial interval between two consecutive attacks may be very short or absent. The intial rigor may be very severe but is generally less marked often amounting to just a chilly sensation only.

The largest percentage of pernicious cases are due to this infection. Pain in the splenic area, bilious vomiting, kidney affection with albuminous urine containing granular and hyaline casts are commonly associated.

Ambulatory cases.

In some cases of malarial fever and these by no means the least dangerous—the subjective symptoms may at first be of so mild a character that the patient may be able to go about his duties with a body temperature of 103° to 104°. These patients may not have any rigor or gastric trouble or any other disabling or distressing symptoms. In some of these, however, the fever is liable to assume a pernicious character. The diagnosis and proper treatment of these cases is, therefore very important.

(ii) Symptoms in detail

Characteristics of pyrexia.

I. *Fever.* Intermittency and periodicity are the characteristic features of tertian fevers. In *P. vivax* infection the temperature rises high very quickly and falls rapidly; the paroxysm is usually completed within 6 to 10 hours and occur synchronously with segmentation at remarkably regular intervals of about fortyeight hours, *i. e.*, every third day (tertian). When the patient is infected with two broods of parasites which reach maturity on alternate days, paroxysms occur daily giving rise to *quotidian* fever due to double tertian infection.

In quartan.

In quartan type of malaria the paroxysms are of comparatively short duration and the temperature usually does not rise above 103° to 105°F., but abnormally high temperatures (107° to 115°F) have been reported. Paroxysms appear every seventy-two hours *i.e.*, every fourth day (quartan fever). The infection may be double or triple, in which case there is a daily paroxysm (quotidian fever); there may be mixed infections with different species which, due to sporulation at various intervals, may produce irregular remittent or continuous fever. In some cases the periods of development of the parasites may not be exactly normal, and the attacks are described as "premature" or delayed. Varieties which are irregular during the first attacks frequently show the cycle later.

In malignant tertain.

In malignant tertian malaria periodicity is difficult to observe. The fever due to the subtertian parasite, is of tertian type, but each attack lasts longer (from sixteen to eighteen hours or more), so that the apyrexial interval between two consecutive attacks may be very short or absent. Although daily intermittent fever (quotidian) is quite common, it may be remittent, continuous, or very irregular. The various stages are less differentiated. The rigor stage is relatively less marked, the pyrexial period lasts much longer. The paroxysm of fever usually lasts twelve to fourteen hours and a second paroxysm often begins before the first has ended.

P. ovale.

The fever caused by *Plasmodium ovale* runs a particularly mild course, and has a tertian periodicity. It has been reported from Uganda, Nigeria, Congo and Cameroons. The attacks begin in the evening or at night, but this discrimination is not always possible. The temperature curve consists of a series of high peaks or narrow bases showing tertian periodicity.

General course.

In maligant tertian fever the primary attack, unless it takes a turn for the worse and becomes pernicious, is of about a week's duration even when untreated. Towards the end of that period the temperature chart shows a characteristic "downward slope".—The first apyretic interval lasts only a few days, and is followed by the first relapse which is often more severe than the primary attack, but lasts about the same time. Thereafter the patient (untreated) usually suffers from four or five relapses of declining severity at intervals of approximately ten or twelve days, when the relapses cease for a considerable time. Afterwards they come on only at irregular intervals and in less severe form. The disease as a whole, is less protracted and is often more quickly cured then is the case with other types.

In untreated benign tertian and quartan fevers the general course is less regular. The duration of the primary attack is very variable, sometimes it comprises only two or three definite paroxysms. Sometimes there is a paroxysm every two days or every three days for as long as two months. After the primary attack the fever-free intervals between the relapses vary between ten days and three weeks. As time goes on, although the relapses tend to recur less frequently, they maintain for a year or more their original severity. This quick disappearance of subjective symptoms after paroxysms is characteristic of malaria, and is very different from the protracted feeling of ill health which follows recovery from even so short an illness as dengue and sandfly fever.

In the great majority of cases of uncomplicated malaria the general course is towards recovery. Persons who are repeatedly infected and reinfected often fail to make a sufficient recovery between their frequent attacks, and they pass into a condition of "Chronic malaria".

Mixed infection.

Mixing of the different malaria species frequently occurs, generally at the begining the asexual development of one species of parasite is suppressed by another and it is only after one species has been eliminated that the other succeeds in appearing. In India mixed infections, benign tertian occurs later on, say in spring, when crescent forms are often still to be found in the blood although in the acute stage the malignant tertian infection (late autumn) predominates.

Anaemia.

Anaemia is due to the destruction of the parasitized red cells, and its degree depends on the severity and duration of infection. It develops rapidly and a peculiar pallor of the skin is associated with it. Napier in a study of 35 hospital cases of different types of malaria reports macrocytic anæmia, the average hæmoglobin in the whole group was 10.67 gm., the mean corpuscular volume was 89.16/U. Hookworm infection was associated with severe cases of anæmia, masking its macrocytic nature. He also reports a positive correlation between the size of the spleen and the Van den Bergh reaction, but none between the severity of anaemia and this reaction. His suggestion is that Van den Bergh reaction is a function of reticuloendothelial cell response rather than of red blood cell destruction. Although all his cases were of the macrocytic type, normocytic anaemia also has been reported by others. There is no hypochromia, for the iron pigment liberated by the destruction of erythrocytes is largely retained by the reticulo-endothelial cells.

In patients with malarial anaemia, administration of quinine produces a rise in the reticulocytes in the blood, which reaches its maximum in 4 to 7 days. This is called '*reticulocyte crises*' and is believed to be specific of malarial anaemia. A sustained submaximal reticulocytosis is thought characteristic of persisting malarial infection.

Splenic enlargement.

During each febrile attack the spleen becomes swollen, painful and tender, and becomes, palpable a few days after the onset. In chronic relapsing cases, especially the ones that

had little or no treatment, the spleen enlarges considerably and becomes hard. Acute splenic pain may be referred to the appendix region. Rupture of spleen as a result of injury may occur.

Enlargement of the liver.

The liver becomes somewhat enlarged and tender, but functional disturbances are uncommon. Jaundice or an icteric tinge is frequently seen. The blood sugar is decreased, suggesting an indirect, general damage of an infective toxic nature to the liver so that it is unable to store glycogen. Indirect Van den Bergh reaction is positive.

Gastro-intestinal disturbance.

During the attack, the mouth becomes dry and the patient feels thirsty, the tongue gets thickly coated, appetite is lost and digestion is often disturbed. The stomach may be displaced due to the enlargement of the spleen. In a large number of cases achlorhydria or hypochlorhydria is produced and in a small percentage of the cases hyperchlorhydria, but the acidity returns to normal after recovery. Nausea with or without bilious vomiting is common. Bowels are usually constipated, but in pernicious malaria, diarrhœa simulating cholera or hæmorrhage from the bowels may occur. Cases with the clinical picture of acute hæmorrhagic pancreatitis are also reported.

Malarial Polyneuritis.

Polyneuritis is not a common complication of malaria. Its occurrence would appear to be determined by the intensity of the infection and certain climatic conditions. It often comes on gradually at the beginning of convalescence. The importance of making an early diagnosis is stressed so that specific treatment may be adopted forthwith.

Eyes.

Hæmorrhages may occur in the conjunctivae as well as in the vitreous humour and in the retina. Malarial amblyopia is usually transient, but rarely persists due to optic neuritis, plugging of retinal and choroidal vessels by parasites or pigmented leucocytes, and consequent multiple hæmorrhages in the fundus. This is easily differentiated from quinine amaurosis, as in this condition there is spasm of the retinal vessels so that the disc is pale and the vessels are shrunken. Quinine amblyopia is more persistent than that produced by malaria.

Circulatory and respiratory systems.

Anginal pains may be caused by malarial infection and it has been said that 4 per cent of malarial cases die of coronary thrombosis. The incidence is higher with Italian or African strains of *P. falciparum,* but with Indian strains severe cardiac complications are not common. Ordinarily the heart is not affected but in severe affections, cardiac weakness and dilatation may occur and give rise to praecordial pain and palpitation; syncopal attacks are not uncommon. The sweating stage of defervescence may be excessive, and cause collapse, and fatal syncope, if the patient rises up suddenly or makes an undue effort.

Malaria is said to produce frequent evidence of myocardial damage. It affects the blood vessels indirectly through its action on the vasomotor nerves and directly by the action of parasites on the vascular tissue cells. Loss of vascular tone and fall of blood pressure may occur possibly due to a lesion of the suprarenal glands. Bleeding from the vessels in different parts may occur, viz., stomach, intestine, brain, skin, etc.

The organs of respiration are rarely affected in uncomplicated malaria. There may be slight bronchitis and dyspnoea may occur in cerebral type.

The skin.

The patient has a pale, and sub-icteric complexion with a tendency to patches of pigmentation. Herpes affecting the lips, mouth, face and frequently the ears is a common concomitant particularly of benign tertian malaria. Urticaria may occur. Durring the pyrexial stage there is often an acute erythematous roseola, and purpuric or petechial hæmorrhages in the skin have been observed.

Urinary system.

During the stage of shivering, the urine is pale and quantity increases, due to the contraction of cutaneous blood vessels. Later when the temperature begins to rise the quantity decreases, the specific gravity is raised, the colour is dark and the acidity is increased, due to increase in metabolism. The quantity is less during the sweating stage; the excretion of urea, chlorides, sulphates, urobilin, urobilinogen and bases, specially potassium is increased during the cold and the hot stages but diminishes during intermissions, though it may still be above normal standards. The phosphates in urine are diminished during the atack, but increase during the intermission. Excretion of iron appears after the actual attack is over and continues for some days. Temporary appearance of serum albumin, proteose and nucleo-proteids has been reported after several attacks. In severe cases, generally subtertian, hyaline and granular casts may appear. Malaria infection is said to produce a nephrosis rather than nephritis in cases with mild urinary changes. Only in quartan cases, a trace of albumin in the urine is definitely associated with depression in plasma albumin. Mild glycosuria, has occasionally been observed in malaria, which disappears with quinine treatment. Bile is present in the urine in cases of jaundice. During convalescence there is polyuria, with low specific gravity and increased excretion of chlorides and potassium particularly in subtertian fevers.

(6) Diagnosis of Malaria

Clinical diagnosis.

The clinical diagnosis of malaria is based on a combination of the following signs and symptoms: (1) Intermittent attacks of fever, the periodicity varying according to the type of infection and the fever coming on with shivering and passing off with sweating. (2) Early and marked anæmia. (3) Splenic enlargement. In chronic malaria the patient may not complain of fever but the tiredness, anæmia (pale yellow complexion) and splenomegaly should arouse a suspicion of malaria. An early and accurate diagnosis is usually possible in a large number of cases but mistakes are not uncommon. Two types of mistakes are commonly made, either a case of malaria is mistaken for some other tropical fever (typhoid, kala-azar, etc) or some other tropical fever as sandfly fever, dengue, etc., may be diagnosed as malaria and when the fever subsides, the erroneous belief of a correct diagnosis may be confirmed. In order to avoid such mistakes, endeavour should always be made to confirm the diagnosis by laboratory examinations.

Laboratory methods.

Smear Examination.—Most important laboratory method of diagnosis of malaria is examination of a blood film, both thin and thick films being examined. The diagnostic points are presence of the hæmozoin pigment free or within the white blood cells. Other subsidiary points of sufficient significance are, monocytosis, basophilia, polychromasia of red blood cells and leucopœnea; pronounced leucocytosis, repeatedly encounterd, definitely goes against malaria. Finding of gametocytes alone does not neccessarily mean that the particular attack of fever is of malarial origin. It may be some other fever superimposed over chronic malaria. On the other hand absence of malarial parasites does not exclude the possibility of malaria. In low grade fevers with enlarged spleen peripheral blood may be negative probably due to the fact that the parasites are mostly in exocrythrocytic stage. There are occasions when even an experienced worker may fail to discover any parasite in a case of malaria. Given to a competent observer, examination of 100 fields of a thin film plus a reasonably careful examination of a thick film will enable a positive diagnosis to be made in 95 percent of the cases of developed malaria, but only 67 per cent of the cases of mixed infection. Malarial parasites in blood films that have been kept for some time may stain imperfectly or not at all. This may account for negative findings in severe and fatal cases of malaria. The age of the film, brand of stain, pH of the buffer are important factors in ensuring good results.

Concentration method.—The concentration method of Bass and Johns is more tedious but the parasitized red cells are effectively concentrated and the red cells and parasites are perfectly preserved and stain as well as in ordinary thin films.

Effect of Anti-malarial drugs.

Since the administration of quinine or atebrin makes the finding of the parasites difficult, the question arises whether or not the drug should be given before blood examination has been made. The present postion with regard to this is, that, where facilities are available the administration of the drug may be postponed till the blood examination has been made, and during this period, the fever may be controlled with ordinary diaphoretic mixtures. This rule, however, is not universally applicable, as it may become dangerous to wait at all if the case happens to be one of malignant malaria. On the other hand, if the conditions are such that the blood examination is not possible or that the findings can not be relied upon, antimalarial drugs should be given at once and thereby confirm the diagnosis therapeutically.

Provocation methods.

Provocation is sometimes employed to establish diagnosis particularly in cases of chronic splenomegaly. The means adopted are: cold or hot applications to the splenic area, exposure of spleen to ultra-violet rays, injection of some nonspecific protein such as milk, or administration of adrenalin by injection, etc. Pentavalent antimony compounds and salvarsan are also used for provocation but are not always successful.

Sternal and splenic puncture.

In atypical cases where routine examination of peripheral blood is negative, especially in subtertian infections, sternal puncture can be employed as a method of diagnosis. In the World War II, many workers used this method and found it valuable. The procedure is quite simple and can be carried out by means of a stout truncated lumber puncture needle. Thick drop preparations can be made from the fluid thus obtained which contain larger number of parasites than peripheral blood. Spleen puncture may occasionally be used as a means of diagnosis especially in chronic and relapsing cases due to *P. vivax*.

Complement Fixation Test.

Antigens prepared from organs rich in malaria parasites or red cells infected with plasmodia as well as prepared from artificial culture of the parasites, are used. The test is performed as for the standard Wassermann reaction. The test becomes positive in about

two weeks and persists for about 5 months. It has been found that 81.6 percent malarial sera give a positive reaction. False positive Wasserman and Kahn reaction for syphillis may occur during malarial attack, persisting upto 30 days after termination of attack. This should be borne in mind to prevent mistakes in diagnosis.

Culture methods.

When clinical signs are suggestive of malaria, but repeated smear exminations are negative, culture method may prove of definite value. Although it requires special laboratory technique, it enables a correct diagnosis in a large number of cases which could otherwise, not be diagnosed. It is particularly helpful in determining the species of the parasite and making diagnosis in cases where quinine has already been administered.

Therapeutic Test.

In addition to a careful record of temperature and clinical examination, the therapeutic test of quinine or atebrin is sometimes helpful in confirming the diagnosis. It may however, be mentioned that a positive test is not so helpful as the negative *i.e.,* if actual administration of any of these remedies for a sufficient period and in sufficient amounts does not reduce the temperature to normal for at least a few days, it is quite logical to conclude that the given case is not one of malaria, provided the drug has been actually taken and absorbed. However, the converse inferences from the positive test will not be justified as quinine may cause fall of temperature in other fevers, or there may be a spontaneous cure. For the purposes of therapeutic test, quinine in doses of 10 to 20 gr a day, or atebrin in doses of 5 grains a day should be given for 4 to 5 days.

Differential Diagnosis.

One uncommon condition that may be confused with malaria is hæmolytic jaundice, and examination of resistance of red blood cells will decide the issue. The resistance is definitely diminished in hæmolytic jaundice, whereas it is normal or may even be increased in malaria. Kala-azar is also liable to be confused with malaria. Marked leucopoenia with absence of marked reduction of eosinophils, plus presence of L. D. bodies either in the blood or in puncture fluids obtained from spleen, liver or marrow, will decide the question. There are many diseases which may show temperature curves like those of malaria, *e.g.* Malta fever, liver abscess, endocarditis, septic diseases, bronchiectasis, hydronephrosis, Hodgkin's disease, leukæmias and many others. A careful clinical examination supplemented by an ordinary routine laboratory examination will enable a correct diagnosis.

(7) Immunity in Malaria

Congenital, racial and acquired immunity.

It can be said in brief that congenital and racial immunity in malaria does not exist; and the acquired immunity is partial and short-lived and exists rather against the toxic substances liberated during the infection, than against the parasite itself. New born babies have been reported to be infected; whether the infection is through the placenta or due to mosquito bites, it shows that infants inherit no immunity from the mother.

In highly endemic areas, 100 per cent of the children have been found heavily infected, with high mortality rate. This has been reported not only in India but also from heavily infected endemic areas in other parts of the world. This epidemiological evidence, too, is against any congenital immunity in children.

Regarding racial immunity, epidemiological data again do not support the existence of such immunity. In India in 1939, it was estimated that there were over 12 million cases of malaria and in Ceylon in the same year, out of a population of 6 million, over 3 million were treated for malaria. Considering that although these races have encountered this disease for centuries and still live in highly endemic areas, not a single child exposed escapes infection. The possibility of any racial immunity is thus excluded. On the other hand, there is enough data to conclude the existence of acquired immunity in adults. There is a low incidence amongst adults in endemic areas, as compared to children below 16 years of age, and waves of epidemics occur in certain non-endemic areas. It is possible that the gradual waning of the immunity, acquired by the population in one epidemic is the major factor in the prevalence of the next.

Mode of production of Immunity

Production of acquired immunity

In a highly endemic area in one race, all infants are infected. Some succumb to the disease, others, due to reinfections and superinfections, gradually acquire immunity, and the spleen and parasite rate falls in adult life. The surviving group at adult age has passed through a continuous process of the selection of the immune, and adults are healthy. The result is that the incidence of attacks in the adults is much lower than in children, indicating antiparasitic immunity. The toxic symptoms are very mild, and patients resume

normal work during intermissions, which points to antitoxic immunity. That a similar immunity is not present in the adult population of a non-endemic area, because of the relative high incidence in adults, justifies the conclusions that the continuance of the highest grade of acquired immunity is dependent upon an adequate and more or less a continuous antigenic stimulation over a long period. This naturally will not decrease favourably the incidence and mortality in a non-endemic area, where the epidemics occur after long periods. In highly endemic areas, although the majority of adults will escape, infant mortality due to malaria will always remain high.

Therefore Sinton as a result of his experiments on rhesus monkeys with *P. knowlesi,* and Wilson on human studies in natural infection, conclude that when infection is rarely acquired, treatment should aim at a racial cure; where exposure to infection is frequent and continued, treatment should aim only at clinical cure of the attacks and should be intensive enough not to interfere with the acquisition of immunity, while clinical prophylaxis is advisable in temporary exposure to frequent infection *i.e.,* short stays in endemic areas.

Mechanism of immunity.

Sinton has shown that successive inoculation with *P. ovale* produces progressively higher degree of resistance to subsequent reinoculation than does a single infection. The same happens in bacterial vaccine inoculations. He is also of opinion that sporozoite infection induces better immunity than trophozoite infection, but Schilling does not agree with that view.

Present view.

The present view about the mechanism of immunity is that two factors are involved, (*a*) antiparasitic element whereby the parasites are kept at such a low level, that no clinical symptoms can be produced. This is noticed in latent infections where an attack develops only in case of sudden lowering of general body resistance. This mechanism is cellular, due to the phagocytic activity of the macrophage cells, which though non-specific and sluggish in their action in the beginning, become very active and specific later on. The macrophages have been found to phagocytose both malarial parasites and parasitized red cells. There is a stimulation and proliferation of the reticulo-endothelial system, for this purpose. The specific nature of the phagocytosis has been worked out by Chopra and others. They have shown that the electric charge on a red cell, particularly with a fully mature schizont, is much lower than on a normal red cell. This is due to chemical changes brought about by the parasite inside the red cell. Both red cells and white cells being negatively charged, ordinarily, the forces of electrical repulsion prevent the two approaching each other. When the red cell is infected and its electric charge is diminished, the forces of repulsion are reduced, and approach and phagocytosis become possible. (b) Antitoxic element is humoral and neutralizes the malarial toxins. The antibodies are probably produced by the macrophage system as a result of the stimulation of ingested parasites. These antibodies are probably opsonin in nature, according to Sinton. There is also an increase of euglobulins in the plasma, while albumin and cholesterol are decreased.

Malarial pigment is considered by some to possesses antigenic powers. The immunity induced by *P. falciparum is* more fleeting than that left by *P. vivax,* and is the most durable in the case of *P. ovale.*

Relapses.

Relapses depend on the treatment carried previously. They occur in regular sequence weekly or after three months. Change of season, fatigue, exposure to cold and heat, wetting, injury, shock, dietary indiscretion, alcoholic excesses, etc., bring about relapses. Relapses frequently occur in the early stage of fevers such as typhoid, influenza etc. The course of a relapse is like a primary attack.

Mortality.

Mortality rate on the whole is not high except in children. Usually it is below 10 per cent but is high in epidemics being as much as 20 per cent or more. Prognosis for recovery for primary attack is excellent even in falciparum if properly treated. Relapse rate is highest in quartan (over 60 per cent), next in benign tertian (over 55 per cent) and least in malignant tertian (over 40 per cent).

2. Chemotherapy of Malaria

During recent years great progress has been made in the chemotherapy of malaria. The following is a classified list of some of the important drugs.

I. *Cinchona derivatives*:—

Cinchona alkaloids *e.g.,* quinine, quinidine, cinchonine and cinchonidine are

all more or less active. Beside these there are alkaloids which occur in minute quantities which are also active *e. g.,* cupreines and cupreidines. The amorphous alkaloids have no antimalarial action.

II. *Amino-quinoline and allied derivatives*:—

Pamaquin (plasmochin or plasmoquine), which has a marked action on sexual forms and action only on the asexual forms of B. T. & Quartan (probably in toxic doses), has no effect on asexual forms of M. T.

Chloroquine (Resochin, Aralen, S. N. 7618)—chloroquine, belongs to the 4-aminoquinoline series. Their action is similar to that of mepacrine and they are said to be more effective; they do not give rise to discolouration of the skin. Chloroquin is said to be more powerful than atebrin. Both Plasmoquine and chloroquine have actions on exoerythrocytic cycle.

Metachloridene (SN, 11,437) is 2 metanilamido—5-chloro-pyrimidine is similar to sulphadiazine. It is active against avian malaria, not so active against human malaria; it may prove active against quartan infection in man.

Pentaquine (SN, 13276) belongs to 8-aminoquinoline series. Its action is similar to that of pamaquin but is said to be too toxic for general use in India.

III. *Acridine derivatives*:—Mepacrine (atebrine) and similar products.

IV. *Sulphonamide derivatives e.g.,* prontosil, sulphapyridine, sulphathiazole, etc., have a destructive action on the plasmodium. Sulphadiazine has a more powerful antimalarial action than others. The sulpha drugs, though they have some plasmodicidal properties, have a number of defects which make them unsuitable for general use in the treatment of malaria.

V. *I. C. I. Series.* Workers in the laboratories of Imperial Chemical Industries took anilinopyrimidine as the basis. Antimalarial activity was encountered in No: 2666 (October 1942) in avian Malaria, but it was not effective against human malaria.

Modifications of its chemical constitution by addition of guanidine chain led to synthesis of No: 3349 (July, 1943). This showed marked activity against avian and human malaria. Hundreds of more compounds were synthesized some of which had powerful antimalarial properties, but were not satisfactory excepting No. 4430 which was quite active; No. 4430 was superseded by 4888 or paludrine which is a double guanidine derivative and is superior to 4430. This compound is not toxic and has marked antimalarial properties in small doses. It has also the unique property of being active against the extra-erythrocytic forms.

(1) Study of Action of Antimalarial Drugs

It will not be out of place to mention here the method by which the efficacy of the various antimalarial remedies is tested.

Avian Malaria Test.

(*i*) *Tests on the parasites of avian malaria—Type 'Proteosoma'.* These tests were used as a routine laboratory practice by Roehl, who adopted the procedure of administering drugs to birds through an œsophageal tube passed into the stomach. The test consists in inoculating intraperitoneally a small quantity of blood from the bird which is being tested, into another bird of the same species. If the inoculated bird does not show parasites within a month, a second bird is inoculated in the same way. Canaries which have passed the isodiagnostic test are infected with a known species of bird malaria (usually *Plasmodium relictum,* Grassi and Feletti) by direct blood inoculation, intramuscularly or intraperi-

toneally. The blood used for infecting the birds is taken when the parasites have reached their maximum number. It may be injected whole or diluted with physiological or citrate saline solution (one drop of blood in two drops of citrate solution). Four hours after infection, treatment with the drug to be tested is begun, and is continued daily for six days. The drug is administered through an œsophageal tube into the stomach, the usual dose being 1 ccm of a solution of known strength per 20 grammes body weight of the bird.

After ascertaining the maximum strength of the solution which the bird is able to tolerate in the dose given, a series of experiments is made to ascertain whether this strength of the drug causes delay in the appearance of parasites in the peripheral blood, and, if so, what further dilutions also cause a distinct delaying effect. The period of delay is ascertained by examining thin blood-films every day, the first day on which parasites can be found being compared with the first day on which they can be found in untreated control birds. In the control birds the parasites generally appear on the fifth day after infection. A drug is not considered to have a distinct therapeutic effect unless the delay in the appearance of parasites in the blood of treated birds is at least extended to the tenth day.

Gameticidal action Test.

(*iii*) *Tests on the parasites of avian malaria—Type 'Halteridium.* The species of parasite commonly used is *Hoemoproteus orizivoroe* which occurs as a natural infection in rice finches from Java and some other Eastern countries. It is a parasite of which only the gametocytes (male and female) can be found in the peripheral blood, the vegetative non-sexual cycle being passed in the endothelial cells of the lungs, the liver and other internal organs. Thus the object of therapeutic tests with this parasite is chiefly to ascertain whether the drug to be tested has a gametocidal action. For this purpose two or three naturally infected finches are selected and after having made a daily count of the parasites during a short period (four or five days), the birds are treated with the drug in the same way as in trials on 'proteosoma' parasites of canaries. The effect of the drug is observed by noting whether the parasites disappear or are greatly diminished in numbers as a result of the treatment.

Monkey malaria Test.

(*iv*) *Tests on the malarial parasites of monkeys.* It has recently been found that several varieties of malarial parasites which occur naturally in monkeys in the Far East and in Africa can be utilised for experimentally controlled therapeutic tests of antimalarial remedies. In particular, it has been found that a species of malaria parasite occurring naturally in monkeys of the species *Silenus irus,* when transferred by blood inoculation to *Silenus rhesus* or *sinicus* causes an acute infection which, when untreated, invariably ends fatally, but when treated with quinine, is cured or can be maintained at a low parasite level for months. In India this method is now being extensively used for evaluating the relative efficacy of antimalarial remedies.

Human malaria Test.

(*v*) *Therapeutic tests on induced and naturally contracted human malaria.* Since the introduction of malaria therapy, it has become possible to study the therapeutics of the disease under controlled conditions and to devise some useful tests for ascertaining the efficacy of preparations for prophylactic and curative purposes. The drugs can be tested either in experimentally induced or in naturally contracted malaria. The antimalarial value of the drug is measured by noting its effects both on the clinical course of the disease and on the malarial parasites in the blood of the patients. It is to be understood, of course, that in all therapeutic tests on induced malaria, every endeavour should be made to imitate natural conditions as closely as possible. An essential condition is that the patients must be infected in the natural way by the bites of infected mosquitoes and not by the direct inoculation of malarial blood.

In general, the only test which can be conducted on patients who contract their infection in the field is a test of the immediate therapeutic value of a short course of the drug to be tested in comparison with the immediate therapeutic value of the same course with quinine.

(2) Study of Cures

(i) Natural cures

Gametocyte appearance.

The life of a parasite depends on the life of the host; if the host dies millions of parasites which are living in it die also. There is a tendency in chronic protozoal infections for a condition of balanced equilibrium to be reached between the patient's power of resistance

(*ii*) Test on *P. gallinaceum* in chick developed by the I.C.I. Laboratories (See under Paludrine.)

and the parasitic invasion. This condition one may term 'immunity' or better 'tolerance.' In malaria the rate of multiplication is decreased by the formation of non-multiplying forms (gametocytes) from merozoites or asexual cycle. In untreated cases of malignant tertian infection, the fever usually subsides as soon as gametocytes appear in the blood. Thus a patient suffering from malaria is, after a fairly long period, cured of the disease without any treatment, provided, of course, he does not get reinfection during this time. Patients who leave the tropics are generally completely cured in about two years, though here and there one comes across cases which persist for five years or more. Natural cures are partially brought about by excessive gametocyte formation which, so to say, converts the asexual cycle to non-multiplying gametocytes. It has been demonstrated that when the asexual cycle of *P. falciparum* is destroyed, for example, by quinine, gametocytes still occur in the peripheral blood; the sexual forms are gradually destroyed, though no further treatment is given.

(ii) Artificial cures

These are produced by the administration of certain antimalarial remedies, which act by destroying the asexual parasites, but have less effect on the gametocytes. The important factors concerned in these artificial cures are:—

Parasite multiplication.

The rate of parasite multiplication.—It is known that the subtertian parasite multiplies at the rate of 24-32 merozoites in 48 hours, and is readily influenced by antimalarial drugs; while the benign tertian parasite forms 16 to 24 merozoites in 48 hours and is not so easily destroyed. A single *P. vivax* produces 24 new parasites in 48 hours, and at this rate of multiplication it can produce 250,000,000 descendants in fourteen days, a number which would produce about fifty parasites per cmm of blood. This is a number which can be detected microscopically but which will not give rise to fever. The next generation however will produce sufficient parasites to cause fever. A remedy which kills 96 per cent of the parasites of the benign tertian form in 48 hours will not cure the disease, for it will only prevent the multiplication of parasites and will not reduce their number. As a matter of fact the rate of multiplication of parasites is somewhat less than that indicated above, because as soon as the multiplication of the parasites is checked, sexual forms appear, and since these can only reproduce in the body of the mosquitoes, they are inactive as far as the human body is concerned. That the difference in the rate of multiplication is not the only factor concerned is evident from the fact that the quartan parasite has the slowest rate of multiplication, *i.e.*, 6 to 12 merozoites in 72 hours yet it is the most refractory to antimalarial remedies.

Parasite Destruction.

Rate of parasite destruction.—The percentage of each brood of parasites destroyed by continuous administration of antimalarial drugs is the other important factor. According to Ross, a man of average weight (10 stones) has about 3,000,000 cubic millimetres of blood and allowing for 5,000,000 red blood corpuscles in each cmm, there will be 15,000,000,000,000 red blood corpuscles present in the body. If there is one parasite per cmm of blood, it means 3,000,000 in the body. The lowest number of asexual parasites causing fever has been calculated to be about 100 per cmm, *i.e.*, 300,000,000 parasites in the total quantity of blood. In severe infections of malignant tertian type the number of parasites is considerably higher, as many as 12 per cent or more of the red blood corpuscles being infected. When the number of parasites falls below 300,000,000 they produce little or no symptoms—'parasitic relapse'. Theoretically a single parasite would in three weeks' time multiply sufficiently to produce fever, and therefore, to effect a cure every parasite must be destroyed. It is also known that in latent malaria, a large number of parasites can exist and multiply in the body without producing any apparent symptoms and any condition which lowers the vitality of the host, *e.g.*, unfavourable climate, fatigue, etc., tends to convert the latent into an active disease.

As regards the rate of destruction, it is known that a single dose of any of the antimalarial drugs will not be sufficient to effect a cure, but a series of doses has to be given. The parasites in the host are not like fishes in a bowl which can be destroyed by mixing a single dose of poison with water. Many of them make good their escape and the drugs have therefore to be given in repeated doses daily, and continued for some time to keep down the parasites and produce a cure. These facts lead us to conclude that rate of destruction must be below 100 per cent. The rate of destruction of the parasites, in order to produce a cure, has been calculated mathematically and found to be over 98 per cent of each generation. It is well known that quinine causes a rapid amelioration of symptoms in all types of fever, and parasites also disappear from the peripheral blood. This effect is probably due to reduction of parasites from over 300,000,000 (febrile stage) to below the number (afebrile stage). Ross found that 8 grains of quinine daily sufficed almost invariably

improve symptoms greatly while it was being taken, but it failed to produce complete ıre even after 6 weeks' administration. Acton found the cure rate in benign tertian fection to be 20 per cent after one month's treatment with quinine.

(iii) Effectiveness of artificial cures

The antimalarial drugs produce an immediate effect which may be followed by partial complete cure. We will now deal with these different aspects of the artificial cure.

(A) The immediate and relative effect of the remedy used.

(B) Partial cures or relapses.

(C) Complete cures or sterilisation.

Disappearance of asexual forms.

(A) *The immediate effects.* The parasiticidal effect is studied by noting the rapidity ith which the asexual forms disappear from the peripheral blood and symptoms are relieved. oo much importance cannot however be attached to the picture presented by the peripheral ood as this gives little indication of what is brewing in the deep-seated foci. In malignant rtian infection schizonts become adhesive and form clumps and so block the smaller ssels of the brain and intestines, giving rise to pernicious symptoms. When quinine is ven the trophozoites disappear within 24 hours after the first dose; gametocytes are ot affected at all, as they have been found to exist for as long as 40 days after commencing eatment, and they are still able to undergo sexual reproduction in the stomach of anophelines. has been demonstrated that if the asexual cycle is destroyed and the patient is put on rdinary tonic treatment (with no quinine whatever) gametocytes that are present either e of senility within a few weeks or are destroyed by the cells of the body, the patient ndergoing a complete cure.

In benign tertian infections the asexual cycle takes place in the peripheral circulation ıd when quinine is given the young trophozoites disappear, within 24 hours after the first ose. The older trophozoites and the developing schizonts are not so easily affected, as ey can be seen up to 48 hours (because sporulation is not completely prevented, and a ight attack of fever is often observed on the third day). The gametocytes disappear from e blood in 4 to 5 days, showing that they are less resistant to quinine than malignant rtian gametocytes.

The method of administration is an important factor in the rapidity of cure; the travenous method is the most rapid in action and next come the intramuscular and the oral utes. If during the course of treatment parasites reappear, it is certain that either the atient is avoiding treatment, or the interval between doses is too long or the alkaloid is ot being absorbed.

Relapses.

(B) *Partial cures and relapses.* The destruction of the asexual cycle by antimalarial rugs is a gradual process of fractional destruction and it is obvious that one single dose r a few doses given at short periods do not suffice to effect a cure. As it is impossible o find out the exact time of liberation of each young brood, the ideal plan appears to be o give a continuous treatment long enough to affect many generations of the parasites 5 to 6 weeks to destroy 10 to 15 generations). Relapses in benign tertian infections, owever, cannot be attributed to insufficiency of treatment, as in some of Acton's experi- ents this was carried on from 3 to 5½ months and in spite of this 50 per cent relapsed. ntimalarial drugs if properly administered, will rapidly relieve the symptoms due to alarial parasites and if properly followed up, will prevent the parasite from causing death r serious damage to the patient. In spite of all the precautions, however, a permanent ure may not be obtained and relapses may occur. What then is the explanation of these elapses? The following possible explanations have been suggested regarding the escape f the parasites from destruction:—

Drug resistance in parasites.

(*a*) *Production of resistant forms.* Against this view is the fact that if antimalarial rugs are properly administered in adequate doses, they cause immediate disappearance f the asexual parasites from the blood. It has often been stated that the first ttack is more easily cured than subsequent ones and this has been proved by experiments ith induced malaria. Investigations by Fletcher (1923) showed that the alleged resistance o quinine in many of the cases during the First Great War was apparent and not real, and hat often it was due to quinine not being swallowed. The drug also may be adulterated. n any case if quinine resistant cases occur they are so few that if scrupulous care is taken n the details of the treatment they may be ignored altogether. Quinine fastness or resis- ance of a particular strain of parasite cannot therefore be accepted as a cause of relapse.

Parasite in deeper tissues.

(*b*) There is another factor which may explain the cause of relapses after treatment. The parasites may take up their abode in the capillaries of the internal organs or in areas where body fluids do not freely reach them, or the patient's powers of resisting the infection (immunity) may not be stimulated. The existence of back-waters, such as the spleen and the bone marrow, where owing to absorption of the drug by the tissues, it does not penetrate and parasites can go on multiplying has been held as one of the causes. In case of malaria in birds it has been shown that the cycle in the blood has an additional schizogony cycle in the reticulo-endothelial cells. These cells act as reservoir from which the blood may subsequently get recompensated. A cycle of development has been demonstrated in the capillaries of brain in birds but not so far in man. However, it has been pointed out that in malignant tertian infections, schizogony occurs in the deep-seated areas, but in spite of this quinine is quite effective and acts as a specific, while in benign tertian where schizogony occurs in the peripheral blood it gives a lower cure rate.

Variations in sensitivity to drugs.

(*c*) *Failure due to different strains of parasites.*—A species may contain many immunologically distinct strains. In addition to immunological differentiation they show differences in other respects, such as severity of the disease manifestations, response to drugs and number of gametocytes produced.

Non-absorption of drug.

(*d*) *Failure due to non-absorption of drugs.* Failure due to non-absorption of anti-malarial drugs owing to catarrhal conditions and inflammation of the gastro-intestinal tract has been assigned as one of the causes.

(*e*) According to new concept the persistency of infections and malarial relapse are closely linked with the cycle of developement of the parasites in the fixed tissue stage or exo-erythrocytic stage. There seems to be little doubt that the cycle of parasite in the human host is far more complicated than was formerly imagined. It appears to be certain now that after inoculation of the sporozoites invasion of the endothelial cells or fixed tissue cells occurs. Within these cells the parasites undergo a distinct cycle of development before they are discharged into the blood stream and the erythrocytic cycle starts.

The essential mechanism of relapse according to this conception results from periodic discharges of tissue exoerythrocytic forms into the blood stream. The persistency of an infection, is an expression of the length of time during which the development in fixed tissues continues. The *P. falciparum* and the *P. vivax* behave differently with regard to their relapse rates and the duration of infection because of this factor. In *P. falciparum* the tissue development stage is short and there is more or less complete expulsion of the parasite from the fixed tissues. In *P. vivax* there is prolonged fixed tissues development and partial repeated expulsion of parasites and this leads to relapses. Even different strain of *P. vivax* may have varying degrees of fixed tissue stages and give rise to different clinical patterns, that is, some are more relapsing than others.

Most of the older antimalarial drugs *e.g.* quinine mepacrine and even the new drug, chloroquine, act practically exclusively on the erythrocytic forms of the older drugs plasmoquin had action on the fixed tissue forms but is toxic; the new drugs, paludrine and pentaquine, act on the fixed tissue forms. It will thus be understood why permanent cure was not affected with quinine and mepacrine and relapses occured.

Conclusive evidence of fixed tissue stage in man is not yet forthcoming but the recent studies point to it. It has been shown that the sporozoites disappear from the blood within half an hour and blood is non-infective if injected into man till parasites appear in the blood. This occurs on the seventh day in P. vivax infection and on the ninth day in P. falciparum, the parasites appearing in the circulation with extraordinary regularity. This preciseness in appearence shows that a uniform periodic cycle is taking place in fixed tissues. Although exo-erythrocytic cycle has not been demonstrated the experience in World War II has forced this conclusion.

Incomplete destruction of asexual cycle.

If all preliminary infections were really cured, the incidence of malaria would diminish. Statistics of the Indian Army show that only 23.5 per cent of the cases coming under treatment in endemic malarial areas are primary infections, while 76.5 per cent are relapses and reinfections.

It has also been pointed out that relapses are much more common in cases of benign tertian than in malignant tertian. The question of individual resistance of the patient is important, as some persons acquire malaria much more readily than others, and this factor also plays an important part in the treatment of malaria. In relapsing cases, all predisposing causes, *e.g.*, excesses of any kind, fatigue, exposure to heat and cold, should be avoided. Transfer to a non-malarial area such as a hill station is advised for persistent cases.

(iv) Complete Cure

Prevention of relapses.

The following factors possibly contribute towards bringing about a complete cure:—

(*a*) *Response of the defensive mechanism of the body.*—The effects of the drugs on the tissue cells of the host, possibly making the erythrocytes noxious, causing the phagocytes to destroy the parasites or perhaps by the formation of antibodies.

(*b*) *Selective action of drugs or erythrocytic and exo-erythrocytic forms.*—By action of drugs both on the exo-erythrocytic and erythrocytic forms of parasites by such drugs as paludrine, plasmoquin and pentaquin.

3. The Cinchona Derivatives

Introductory.

The Cinchona alkaloids occur in the various species of two rubiaceous genera, *Cinchona* and *Remijia*, which are indigenous to the eastern slopes of the Andes in South America between the latitudes 10°N and 20°S. Cinchona comprises about forty species of evergreen shrubs or trees, which grow isolated or in small clumps in dense forests. The average height at which they flourish is an altitude of 3,000 feet to 10,000 feet above the sea level. Some of the species are found as high as 11,000 while others as low as 2,500 feet. The trees may reach the height of 80 feet and are valued for their bark, which contains certain potent alkaloids.

Cinchona bark was first brought to Europe in 1640 and was used in the treatment of ague. As early as 1866 cinchona plantations were started in the Nilgiri Hills, the species growing best being *C. officinalis.* Plantations were also established in the Himalayas, Sikkim near Darjeeling, and in the Karen Hills in Burma. The species growing best there were *C. succirubra* and *C. calisaya.*

Species of cinchona.

Of the large number of species and sub-species only a few yields commercially valuable barks. They are:—

I. *C. officinalis* var. { *condaminea*, *bonplandiana*, *crispa* } yielding crown barks.

II. *C. succirubra* (Pavon), yielding red bark.

III. *C. pitayensis, etc.*, *C. lancifolia*, *C. cordifolia* } yielding Columbian red bark.

IV. *C. nitida*, *C. mircantha*, *C. peruviana* } yielding grey bark.

V. *C. calisaya* yielding yellow bark.

Newer Plantations.

The successful transplantation of cinchona was followed by a period of researches and experiment to find the proper ways and means of propagating the new plants. In Java all the varieties were found to possess a low percentage of quinine with the sole exception of *C. officinalis;* this variety however did not grow very well. The Calisaya varieties gave the highest yield of quinine, the average quinine content being 6 per cent, exceptional samples yielding from 10—12 per cent. The plantation of this variety was therefore taken up, and large plantations were started by the Government as well as by enterprising private individuals. Cinchona cultivation flourished in Java and at present about 9 to 10 million pounds of quinine are produced annually on an average; this may be estimated from the fact that about 90 per cent of the quinine supply of the world comes from that island. The maximum content of the alkaloid occurs in trees between the ages of seven and eleven years. *C. succirubra* has proved the hardiest and most easily cultivated species, and it gives a high yield of total alkaloids (10 per cent), in which quinidine and cinchonine content preponderates over that of quinine. In the hybrids, *C. officinalis* and *C. robusta* the total alkaloidal content is very large, and of late years the quinine yield has also considerably increased.

Cupreæ cortex contains both cupreine and quinine and is obtained from *Remijia pedunculata* and other species.

Cinchona Alkaloids.

The therapeutic effects of cinchona bark are due to the crystalline alkaloids present; of these *quinine* is the best known, while *quinidine, cinchonine* and *cinchonidine* have been used to a comparatively less extent in medicine. The alkaloids are contained in the cellular tissue of the phloem. In the leaves they are only present in small quantities but larger quantities are found in the stem bark increasing from above downwards as the root is approached. The alkaloids exist in the bark in combination with cinchotannic acid and quinic acid. The former is oxidised by exposure to a red colouring matter which is present in large quantities in *C. succirubra.*

The bark is usually collected by felling the trees or by 'capicing'. These methods are now largely employed in preference to other methods and the plantations are so arranged that every year a large area is matured and the trees are ready for cutting down. The trees yield the maximum amount of alkaloids when they are 6 to 9 years old and it is about this time that they are cut down. In Russia the plantation is allowed to grow for 2 or 3 years and it is then cut down and alkaloids are extracted. The alkaloidal content by this method is low, being not more than 1 to 2 per cent.

Composition of bark.

Composition of Cinchona bark.—Pelletier and Dumas in 1820 isolated quinine from the bark, and later quinidine, cinchonine and cinchonidine were isolated. In addition to these four alkaloids the bark contains many more alkaloids; these are divided into crystallisable and amorphous.

The bark in addition to these alkaloids contains certain acids, neutral principles, colouring matter and traces of a volatile oil, gum, starch and other vegetable matter. The ash 1 to 2 per cent consists mainly of calcium and potassium carbonates and a little silica.

Extraction of alkaloids.

Extraction of Cinchona alkaloids. The principal dried barks used for the production of quinine are:—*C. succirubra* (red cinchona bark), 1,000 gm of good bark yielding at least 50 gm of total alkaloids containing 15 gm of quinine sulphate; *C. calisaya* (yellow cinchona bark) which with the same amount of bark yields 60 gm of total alkaloid containing 30 gm of quinine sulphate; *C. officinalis* (crown or Loxa bark) containing 5 per cent of total alkaloids, of which $3\frac{1}{2}$ per cent is quinine; the bark of *C. lancifolia* or Columbian bark contains about 2 per cent of the alkaloids with only a small proportion of quinine.

The bark is dried and ground to a fine powder mixed with slaked lime, which is passed through a seive of 40 meshes to the linear inch, sufficient water being added to make it damp. The addition of lime makes the subsequent extraction of the alkaloids easier. The mixture is made into a thin paste with water and put into vats. Caustic soda and warm paraffin oil are then added and the vats heated to 170°F. with frequent stirring of their contents; these are then allowed to settle when two layers form, the oil on the top containing the alkaloids and the exhausted bark below. The oil is decanted off and mixed with dilute sulphuric acid which dissolves all the alkaloids. The mixture on standing separates into two layers, the acid liquor containing all the alkaloids in solution. The alkaloids are then precipitated by adding caustic soda. The crude total alkaloids thus obtained, sometimes called cinchona febrifuge are further purified.

(1) Total Alkaloids, Totaquina

Total alkaloids of bark.

Totaquina.—The fact that the alkaloids of cinchona, other than quinine, possess therapeutic activities almost as great as quinine itself has increased the possibility of using total extracts of cinchona bark. Such extracts, which are obviously less expensive than the purified alkaloids, were first prepared in India in 1874 from the red bark of *Cinchona succirubra,* in which quinine constitutes about one-third of the alkaloids present. This powder was called quinetum or febrifuge. By 1903 trees of *Cinchona succirubra* had become rare in India as attention had been focussed on the cultivation of *C. calisaya* and *C. ledgeriana* and their hybrids, species in which the relative proportion of quinine to the other alkaloids is about 70 per cent. *C. calisaya* and *C. ledgeriana,* though they can be raised with care in the eastern Himalayas, grow well in Bolivia and in Java. Cinchona febrifuge was therefore manufactured from the alkaloids which remained after the extraction of quinine from yellow bark, some quinine being added to make it more or less similar in composition to the original febrifuge. It is, therefore, not surprising that somewhat variable results were obtained in the treatment of malaria with this relatively unstandardised febrifuge. In order to overcome this difficulty the Malaria Commission of the League of Nations (1932) introduced an improved and standardised mixture known as "Totaquina". This mixture contains 70 per cent of crystallisable alkaloids and not less than 15 per cent

of quinine; on the other hand, it should contain not more than 20 per cent of amorphous alkaloids and not more than 5 per cent of water, while the mineral ash must not exceed 5 per cent and, if possible, 3 per cent. Two types of totaquina are allowed: Type I is prepared from the total alkaloids of *C. succirubra,* and it is to be produced on a large scale it will necessitate the extensive planting of trees of this species. Type II, in which there is as a rule more cinchonine than in Type I, is prepared from *C. ledgeriana* residues with the addition of sufficient quinine to bring it up to specification. Although there have been from time to time complaints that totaquina is not completely soluble in an acid mixture and undoubtedly complete standardization of composition cannot be obtained, nevertheless there is general agreement that totaquina acts like quinine as a potent remedy in all forms of malaria. Hicks and Chand (1935) in India found that both types of totaquina were equally efficacious provided daily doses of 0.6 gm per 70 kilo of body weight were given in benign tertian and 1.2 gm per 70 kilo of body weight in subtertian infections. While totaquina possesses no advantage therapeutically over quinine and is, if anything, slightly less efficient, nevertheless its cost of production is so much lower, that its use is obviously indicated in countries such as India, where at least 100 million persons a year suffer from malarial infection. Since the adoption of totaquina by the British Pharmacopoeia (1933) its use has become wide spread. In the Philippines, as in India, an excellent totaquina of type I has been prepared from the total alkaloids extracted from the bark of either *C. succirubra* or *C. robusta.*

Totaquina two types.

Efficacy of totaquina.

The composition of the two types of cinchona febrifuge (Totaquina) is given below:— *Composition.*

	Totaquina Type I. Made by extracting the total alkaloids from cinchona bark: (C. succirubra)	Totaquina Type II. Addition of sufficient alkaloids to residue of quinine manufacture.
Quinine	25.23 per cent	14.79 per cent
Cinchonine	27.67 „ „	55.11 „ „
Cinchonidine	34.13 „ „	7.10 „ „
Quinidine	0.0 „ „	6.21 „ „
Total crystallisable alkaloids	87.03 „ „	88.21 „ „
Amorphous alkaloids	8.86 „ „	10.47 „ „
Moisture	0.99 „ „	1.77 „ „
Ash	0.63 „ „	2.13 „ „

(2) Alkaloids of Cinchona

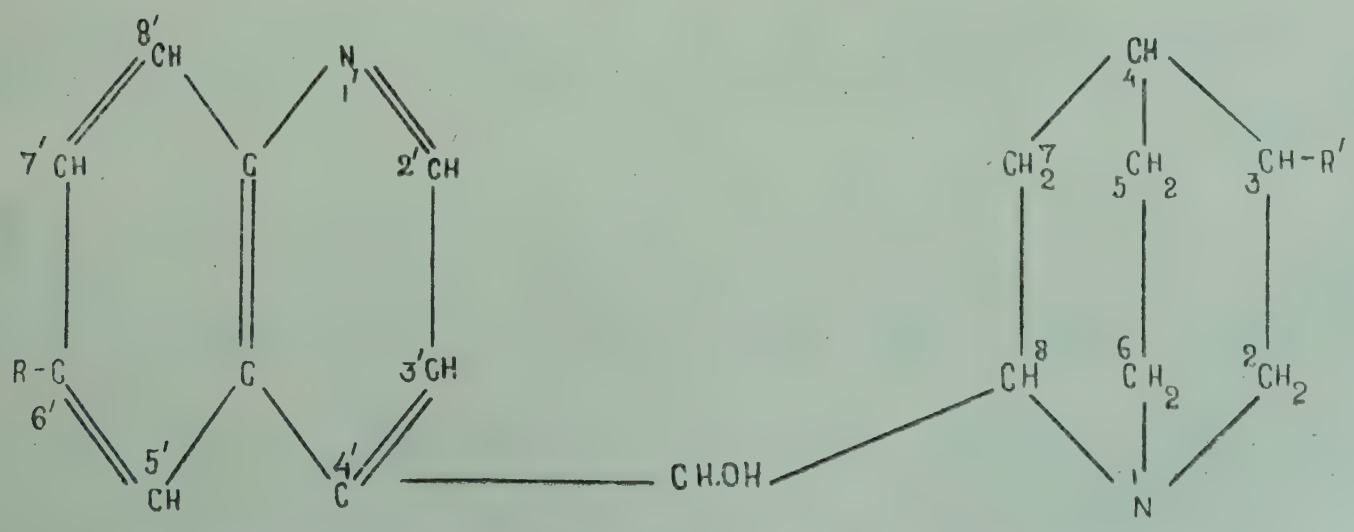
The four chief crystallisable alkaloids derived from cinchona bark are, quinine quinidine, cinchonine and cinchonidine, but over twenty other alkaloids mostly amorphous have been isolated from various species of cinchona and cuprea. These alkaloids are sometimes described collectively as quinoidine though strictly speaking quinoidine is the pitch-like residue left after removal of all the commercially valuable alkaloids from the total alkaloids of cinchona bark.

Cinchona alkaloids.

The four alkaloids, quinine, quinidine, cinchonine and cinchonidine, form two pairs of isomerides of which each member of the first differs from each member of the second by the residue of a methoxyl group—CH_2O.

Constitution.

The following general formula is now usually assigned to this group of alkaloids (cf. Rabe, 1909; Rabe, Huntenburg, Schultze and Volger, 1931).



Quinoline group—Secondary Alcohol group—Quinuclidine group

In quinine and quinidine R=OCH_3.
R'=$CH:CH_2$.

In cinchonine and cinchonidine R=H;
R'=$CH: CH_2$.

In cupreine R=OH.R'=$CH:CH_2$.

In the hydro bases R' becomes $CH_2.CH_3$.

In the alkylcupreines R becomes OAIK (homologues of quinine).

In the alkylhydrocupreines and alkylhydrocupreidines R becomes OAIK and R' becomes $CH_2.CH_3$ (homologues of dihydroquinine and dihydroquinidine)

d & l rotatory alkaloids.

It is possible to divide the cinchona alkaloids into two series, a dextrorotatory and a laevorotatory:—

Chemical Name	Natural Alkaloid	Corresponding hydroalkaloid
	Cinchonine Series—Dextrorotatory Alkaloids	
Cinchonine	Cinchonidine	Hydrocinchonine
Hydroxycinchonine	(Cupreidine-unknown)	Hydrocupreidine
		Hydroquinidine
Methoxycinchonine	Quinidine	
	Cinchonidine Series—Laevorotory Isomerides	
Cinchonidine	Cinchonidine	Hydrocinchonidine
Hydroxycinchonine	Cupreine	Hydrocupreine
Methoxycinchonidine	Quinine	Hydroquinine

Substitution products.

Modification of the cinchona alkaloids gives rise to a number of compounds of therapeutic interest. Hydrogenation of the vinyl group and by replacement of the methoxyl group of quinine and quinidine by higher alkyloxyl groups the following compounds are produced. When the Alkyloxyl group is

OCH_3	.....	.....	Methylhydrocupreine is formed.
OC_2H_5	.....	.....	Ethylhydrocupreine (Optochin) is formed.
$OCH(CH_3)_2$	.....	.....	*iso* Propylhydrocupreine is formed.
$OCH_2CH(CH_3)_2$	.....	.....	*iso* Butylhydrocupreine is formed.
$OCH_2.CH_2.CH(CH_3)_2$	.....	.....	*iso* Amylhydrocupreine (Eukupin) is formed.
$O.(CH_2)_5.CH(CH_3)_2$	.....	.....	*iso* Octylhydrocupreine (Vuzin) is formed.

Optochin has a curative action in pneumococcal infections in mice; vuzin is toxic in vitro to Corynebacterium diphtheriae, while eukupin and vizin possess local anaesthetic properties.

(3) Therapeutic Efficacy

Therapeutic efficiency.

While the introduction of atebrin-like compounds and plasmoquin represents a notable advance in the scientific treatment of malaria, quinine still ranks high in current practice, on account of its clinical effectiveness, low toxicity and the widespread knowledge of its use and dosage.

Of the natural cinchona alkaloids, very active in curing infections due to *Plasmodium inconstans* in canaries, was dihydroquinine, followed by quinidine, quinine, cinchonidine and cinchonine in descending order. The effect of hydrogenation has been shown to vary greatly for different alkaloids. For quinine, and possibly quinidine, hydrogenation increases the activity, while for cinchonine the effect is probably to diminish the activity.

No obvious relationship can be traced between optical activity and anti-malarial action in the cinchona alkaloids.

The anti-malarial activities of the alkylquitenines have been investigated. On oxidation

of quinine the inactive acid quitenine is formed. Activity is restored to quitenine and the other analogous carboxylic acids derived from quinidine, cinchonidine and cinchonine by esterification. As the molecular weight of the alcohol used for esterification increases, so does the anti-malarial activity, the maximum being reached at butyl or amyl quitenine, after which there is a fall.

It is obvious that at present no precise general conclusions can be drawn as to the mode of action of the cinchona alkaloids as anti-malarial drugs. It is, however, generally agreed that the reticulo-endothelial system may play some part in the process (cf. Kritschewski and Demidowa, 1934).

(4) Quinine Supply of India

The number of individuals who suffer from malaria every year in India, has been conservatively estimated at a minimum of 100,000,000. For the purpose of mass treatment the amount of quinine should be the minimum effective dose which would relieve the symptoms. Taking this at 45 grains, a rough approximation of the annual requirements of quinine for India would be 100,000,000 x 45 gr or approximately 600,000 lbs. The amount of quinine used in India is approximately 200,000 lbs. If the above figure (600,000 lbs.) of annual requirement of India be taken as correct (although the maximum figure given is 1,250,000 lbs), the present consumption in India is less than a third of the actual requirement

Annual requirement.

As regards the question of production of quinine, India still produces less than half the amount of quinine annually consumed by her population. Out of the total of 200.000 lbs 110,000 lbs are imported and only 90,000 lbs are produced in India. (Figures according to Krishnan are the amount imported 140,000 lbs, the amount produced 70,000 lbs).

Complexity of the Problem.

The provision of adequate treatment for the malarious sick in India is a difficult problem. Although it has been established that all the crystalline alkaloids of cinchona have a remarkable anti-malarial action, the importance of the fact has not been generally realized. If the total alkaloids begin to be used in general practice the treatment of malaria could be made much cheaper and could be extended among the general masses.

Extension of Cinchona Plants.

In addition to this the question of extension of cinchona plantations has also been considered. One of the arguments brought up against the extension is that in view of the rapid development of the synthetic anti-malarials there will be no further necessity for cinchona alkaloids. The author considers that unless something extraordinary happens during the next 15 to 20 years, India will have to rely mainly on the cinchona alkaloids for the struggle against malaria. The people in India prefer cinchona alkaloids to mepacrine and similar compounds. Whether the new drug paludrine (4888) will replace cinchona alkaloids completely remains to be seen.

Under the present conditions in this country, therefore, it would be very desirable to extend the cinchona plantations in India. As regards the scope for increasing the production of quinine in India, according to Krishnan, the number of acres of land available for cinchona cultivation in India is 38.000 which can produce 6,840,000 lbs of quinine.

4. Pharmacological Action of Cinchona Alkaloids

To comprehend the rationale of the therapeutic action of these alkaloids it is important to have a clear idea of their general pharmacological action. The type of action produced by quinine closely resembles that produced by the other alkaloids and it can be taken as a typical example of this gronp.

Action on protoplasm.

The action of quinine is specially marked on leucocytes, amœbæ and spermatozoa. The action generally begins with temporary stimulation of activity of the protoplasm, being quickly followed by inhibition of all vital processes and finally death. Quinine probably forms a film on the surface which decreases the permeability of the cell, thereby producing a narcotic action such as is seen on local application to nerve cells, muscle cells, etc. All these effects give this alkaloid the property of being a general protoplasmic poison. Its toxicity to vertebrates is however comparatively low. The metabolic processes in the protozoal organisms are retarded. Minute doses therefore merely produce slowing of the activities in the organisms, while large doses produce first paralysis and then death. Very dilute solutions such as 1 in 20,000 to 50,000 destroy amœbæ and paramœcia in a few hours and some pathogenic protozoa, *e. g.*, plasmodia. The protoplasm in the amœbæ becomes granular, spherical and in a few hours nothing but granular detritus remains; this action occurs in the plasmodia in presence of the merest traces of quinine.

Bacteria.

The toxic action of quinine on the protoplasm endows it with bactericidal power but the action of the alkaloid on cocci and bacilli is somewhat varied. Its germicidal efficiency is considered to be about half that of phenol, whilst its antiseptic action approaches that of mercuric chloride. A dilution of 1 in 2,000 delays growth of bacteria and 1 in 800 inhibits the growth in fluids of organic matter and prevents lactic and butyric acid fermentation. The drug also retards the action of many unorganised ferments.

Some organisms are very sensitive to quinine and its derivatives while it has little or no action on others. Moulds are most resistant to it, in fact, they thrive in solution of quinine sulphate. About 0.5 per cent is required to stop the growth of putrefactive bacteria and 2 per cent to kill them. Typhoid bacilli cannot grow in 1 in 30,000 solution. Quinine has no direct influence on the course of most bacterial infections whether given by injection or otherwise.

Some of the newer derivatives of cinchona, for example the cupreines, exert a powerful bactericidal action on certain micro-organisms even in the presence of proteins, and at the same time have a relatively low toxicity for the body cells. This action as a rule increases as we go up the series from ethyl hydrocupreine to iso-octyl-hydrocupreine. Thus quinine in 1 in 1,000 concentrations, ethylhydrocupreine in 1 in 2,500 and iso-octylhydrocupreine or vulin in 1 in 60,000 concentrations, will destroy *B. tetanus*. The same is true of streptococcus, staphylococcus and *B. diphtheria* with some reservations.

Ethylhydrocupreine has a specially powerful action against experimental pneumococcal infection, but unfortunately it has toxic action on the optic nerve and produces blindness even in moderate doses. Iso-octylhydrocupreine has a powerful action against streptococci and tetanus in septic wounds in concentration of 0.1 gm in a litre of water.

(1) Action in Man

External action.

Quinine solutions applied to the unbroken skin do not produce any disagreeable sensation. Rashes are however frequently produced in workers in quinine factories after prolonged exposure to the drug and certain local irritation is set up. Quinine and its salts are not absorbed by the intact skin but are taken up rapidly by the mucous membrane.

Alimentary system.

When taken by the mouth quinine acts as a bitter, stimulates the gustatory apparatus and improves the appetite; the peptic glands are reflexly stimulated. In the stomach it is dissolved by the hydrochloric acid but it has no direct action on the flow of the gastric juice. Given by the mouth there is little difference between soluble and insoluble salts, as the alkaloid is converted into soluble hydrochloride and the less soluble salts are made more soluble by the gastric acid. Passing into the duodenum, it is usually absorbed rapidly. The absorption occurs mainly from the small intestines and it is rapid and complete during the first six hours after administration of a dose. Absorption is retarded if quinine is given with or soon after food. The soluble salts of the alkaloid are absorbed a little more quickly than the insoluble ones, for compounds like euquinine and quinine tannate have first to be hydrolysed by the alkali of the duodenum before they are absorbed.

Digestive enzymes.

Various digestive enzymes are affected to varying extents according to the degree of concentration of the alkaloids. The activity of ptyalin, the starch digesting ferment of the saliva, is inhibited by the acid salts in vitro. As the digestion of starches is over in 10—30 minutes, no interference will result if the alkaloids are given half an hour after a meal. Peptic inhibition is more marked with the cinchonine series and the inhibition is less when the ferment first attacks the protein than when the protein is first acted upon by the alkaloids. On the tryptic digestion, quinidine has a more powerful inhibiting action than quinine; the action of pepsin is retarded in the presence of a 1 in 5,000 solution of quinine or quinidine.

The deduction of practical importance is that, the time of administration of these alkaloids in relation to meals is of the utmost importance. They should be administered when they least interfere with the activity of the digestive enzymes, that is about 1½ to 2½ hours after meals.

Circulatory system.

Action on the blood: The leucocytes lose their amœboid movements, become round and granular, diapedesis through the capillary walls ceases and the coagulation time of the blood is reduced. Even in very low concentrations (such as those occurring in the blood after administration of therapeutic doses of quinine) the movements of the leucocytes are inhibited and diapedesis is stopped. This can be demonstrated in the exposed mesentery of the frog.

Erythrocytes. On the erythrocytes, the acid salts of quinine have a hæmolytic action *in vitro,* and blackwater fever has been attributed by some to the toxic effects of quinine salts on the red blood cells. Quinine circulates in the blood as quinine base. Acton has demonstrated that strong solutions of quinine and quinidine base such as 1 in 2,000 in normal saline, produce no hæmolysis of washed red corpuscles *in vitro,* even after incubation for 24 hours. In the case of acid salts, such as the bisulphate, there is faint hæmolysis in 1 in 8,000 solution, but none whatever in 1 in 11,000 dilution. Intravenous injections of strong solutions of acid salts are therefore highly undesirable and there is no doubt that rigors, which are sometimes observed after such injections, are due to the hæmolysis thus produced.

Myocardium.

Action on the heart. The heart muscle is acted on in the same way as the other muscular tissue. Very small quantities accelerate the pulse and raise the blood pressure; large quantities produce the reverse effect, the action is directly on the motor apparatus of the heart and not on the nerves. The depression of the heart is responsible for the collapse produced by very large doses in patients suffering from fever. Quinine depresses isolated strips of the heart of a turtle. A frog's heart is weakened by 1 in 50,000 solution, and 1 in 5,000 stops the heart in diastole in a few minutes (large doses of digitalis stop the heart in systole).

Quinine is considered by some authorities to be the greatest regulator in arrhythmia due to extra-systole or auricular fibrillation. It has been combined with digitalis in cardiac diseases for over a century. Quinine and digitalis are given not only where arrhythmia is present, but also where large doses of digitalis seem urgently needed and where concomitant disturbances make quinine desirable. Quinidine, a dextro-rotatory isomer of quinine, has lately been the subject of much study owing to the beneficial effects it produces in auricular fibrillation and arrhythmia. The relief afforded by quinine is believed to be produced by the depressant effect of the alkaloid on the heart muscle which prolongs the refractory period of the auricular muscle, in this way ending the circus movements. This reduces the excitability of ventricles and auricles to a point below that at which fibrillation is possible. Most of the cinchona alkaloids lower the contractibility of the cardiac muscle.

Blood pressure. The blood pressure is definitely lowered after intravenous injection and Acton has pointed out that this depression is much more marked with the dextro-rotatory cinchonine series, quinidine being the worst offender in this respect.

Respiration.

Given intravenously, these alkaloids have a depressing effect on the respiratory centres. Hydrobromides have a much weaker effect in this respect than the hydrochlorides and therefore these are preferred for intravenous administration. It has been shown that after lethal doses in cats (25 mgm per kilo body weight) the heart continues to beat for two minutes after stoppage of respiration.

Central nervous

On the cerebrum, quinine has a depressing effect, though not to such a marked degree as some of the other antipyretic drugs. Because of its sedative action on the central nervous system it has been used to induce sleep, a few grains being given at bed time. Large doses may produce mental excitement such as that manifested in cinchonism. When taken for prolonged periods it may give rise to psychical derangements. In rare cases there is loss of consciousness accompanied by delirium, convulsions, stupor, coma and death. Weakening of the heart is the main cause of death, but respiration stops before the heart. Death has been recorded with a 2 gm dose but as a rule fatal dose is 8 to 10 gm or more. As quinine is rapidly excreted, recovery generally takes place, but deafness and defect in the sight may remain for weeks or even months. In ordinary therapeutic doses it has no effect either on the medulla or on the cord, but large doses depress these structures and the respiratory centre is especially attacked.

Peripheral

The cinchona alkaloids and their derivatives produce a slow and prolonged abolition of sensation by their direct effect on the sensory nerve endings. For this reason quinine is not infrequently employed in the treatment of neuralgia. While 1 in 50 solution of

cocaine hydrochloride will produce anæsthesia of the cornea of a rabbit, the same effects can be produced by 1 in 60 of quinine hydrochloride, 1 in 100 of hydro-quinine, 1 in 1,000 of ethyl hydrocupreine (optochin) and 1 in 1,200 of iso-amyl-hydrocupreine. Quinidine and cupreidine compounds have also great anæsthetic properties but their action is somewhat weaker.

Quinine is often combined with urea and is used as a local anæsthetic. This combination increases its solubility and its power of penetration into the cells, but the resulting compound is decidedly weaker and has to be used in twice the strength necessary if quinine alone is used. As a rule 0.25 to 1.0 per cent solutions are quite effective. The disadvantages of the cinchona derivatives in comparison to cocaine and its allied compounds are that (1) injections are painful; (2) the induction of anæsthesia takes longer; (3) they are more irritating and in stronger solutions may cause sloughing; (4) the anæsthesia produced may last for several days.

Special senses. The eye. Quinine and its allied compounds, when given in toxic doses, produce visual disturbances such as contraction of visual fields, diminution in acuity of vision, colour blindness leading to amblyopia and amaurosis. The development of the blindness varies enormously, some patients quickly become amauritic, whilst in others varying degrees of amblyopia supervene. The pupils are dilated and of sluggish reaction; there is central scotoma for green and red. Ophthalmoscopic examination of such cases shows constriction of the choroidal vessels; the retina looks pale and the disc hazy and œdematous. The constriction of blood vessels is probably of vaso-motor origin and it is much more marked with such derivatives as optochin; later the vessels become dilated. Thirty grains of quinine taken in one dose may bring about this condition but therapeutic doses as a rule produce little effect. With large doses temporary and permanent blindness have been known to occur, probably from the effect of the drug on the nerve fibres of the ganglion cells. Generally a speedy recovery is to be expected, but in many patients the central vision is permanently damaged and in addition the light sense remains defective and the visual fields remain contracted. Colour vision may be considerably regained; a certain amount of dilatation of the pupils may remain. It is believed that most of the visual disturbances in patients taking ordinary therapeutic doses of quinine in malaria are due to parasitic emboli in the retinal vessels and not to the effect of quinine on the retinal elements. Treatment of such patients consists in giving strychnine which is a direct stimulant to the optic nerve fibres, and inhalation of amyl nitrate and oral administration of 2 grains of sodium nitrite daily, with a view to dilating the blood vessels.

Special senses.

The ear. The common accompaniments, ringing in the ears and deafness appear to be chiefly due to congestion of the blood vessels. Sixty grains in 24 hours may cause marked disturbances in the ear and even deafness. In rabbits congestion and ecchymosis in the internal ear were produced by giving large doses of quinine. Degenerative changes have also been noticed in the cochlear ganglia; otitis media may be set up by long-standing congestion, and permanent deafness may be produced. When giving quinine to patients with middle ear disease, the possibility of its activating a quiescent condition should be borne in mind.

Quinine has a specific action upon the special sense organs, the first sign of an overdose being ringing and roaring in the ears, accompanied by slight deafness. At the same time the eyes are affected, and there is diminution in the field of vision, photophobia and even temporary blindness. The effects are due to the action of quinine on the nervous elements in these organs.

Metabolism.

The metabolism is affected in a most remarkable manner by quinine in very small doses. Interference with the activity of ferments is a well marked property of quinine and its inhibiting action on oxydases in the blood is well known. Freshly excised still-living kidneys, perfused with blood containing glycocoll convert benzoic acid into hippuric acid; if however minute traces of quinine are added to the blood this synthesis is prevented. It seems likely that the chief effect of quinine on the living organism is to restrict many of the normal chemical changes—synthesis, oxidation and decomposition and in this way to diminish the metabolism (Schmideberg). These effects are probably due to the paralysing action of quinine on the intracellular enzymes by whose aid the chemical changes are brought about. All the metabolic processes in the body, whether of anabolic or katabolic nature, are inhibited and tissue waste and energy production are both retarded. There is a slight transient increase in the nitrogenous constituents of the urine followed by a marked decrease, especially in urea and uric acid. Quinine is said to cause diminution of heat-production by decreasing metabolism from its action on the muscles and glands. It inhibits both the anabolic and katabolic processes of the cell, the processes of oxidation being thus markedly reduced. For this reason, economy in the metabolism of the body as a whole is secured,

especially in those conditions in which katabolism has been stimulated by pathological processes. After administration of quinine in febrile conditions, urea, uric acid, sulphates, phosphates and chlorides decrease in the urine. The use of quinine therefore in wasting diseases such as tuberculosis, is said to be based on rationalism as it tends to check the excessive breakdown of proteins which occurs in these diseases. It is also significant that in spite of the fact that metabolism is diminished, the gaseous interchange, *i.e.*, the absorption of oxygen and the elimination of carbon dioxide remains unaffected. This means that some nitrogenous food, which normally is used up and later appears as a solid constituent of the urine is being stored in the body and the animal, being in equilibrium, is putting on weight.

Effect on the body temperature.

In healthy individuals quinine produces no effect on the body temperature, but in most febrile diseases ordinary therapeutic doses of quinine produce a fall of temperature. It would seem natural to connect this antipyretic action with the depressing influence of the drug on metabolism which would thus produce diminished production of heat. But against this view is the fact that oxygen intake and carbon dioxide output are not materially altered. It has been shown, however, that oxidation is not the only source of heat production and that heat may be produced by the splitting and hydration of nitrogenous molecules, in the course of which nitrogen is converted into urea. In febrile animals the body temperature falls after quinine, even when all the increased loss of heat is prevented. Calorimetric experiments show that loss of heat from the body surface is diminished after quinine. The only possible explanation could be that the antipyretic effect is due to diminished production of heat. The action of quinine in this respect is the reverse of other antipyretics, such as those derived from the coal tar. The fall of temperature runs a course parallel to the nitrogenous metabolism, as measured by the excretion of nitrogen in the urine.

Effect on the muscle tissue.

Quinine first causes a temporary increase and then a decrease in the absolute strength and the working capacity of muscle and finally death followed by immediate rigor. Dilute solutions hasten fatigue. The results are the same whether a muscle is curarised or not; the action is therefore on the muscle fibres, irrespective of their nerves. On smooth muscle the effect is similar. The total effect on muscle tissue with therapeutic doses is small.

Uterus.

Quinine has often been employed to increase the force of contractions in the second stage of labour, under the belief that it favours the contractions of uterine muscle; it is also believed that given in large doses it has an ecbolic action. The action appears to be directly on the muscle fibres without the intervention of the nervous system, as atropine does not modify its action. According to some observers quinine inhibits the non-gravid uterus and stimulates the gravid uterus, because the distension of the fibres has rendered them sensitive to any kind of stimuli. It should be remembered that high temperature, which often accompanies malaria may in itself produce death of the fœtus and evacuation of the uterus. Temperature in pregnant women suffering from malaria should not therefore be allowed to go above 103°F. Experiments on isolated pregnant uteri of rats and guinea pigs show that in dilutions of 1 in 300,000 quinine produces no effect whatever, 1 in 150,000 only produces contraction under certain circumstances and 1 in 44,000, a concentration which can never be attained in the blood unless the patient is literally poisoned with quinine, produces a tonic spasm of both the circular and longitudinal fibres, which if maintained kills the fœtus. Dilution of 1 in 10,000 to 1 in 5,000 may produce relaxation and paralysis. Concentrations of 1 in 150,000, such as occur in blood normally after ingestion of quinine in moderately large doses, stimulate the intermittent contractions. If now some exciting cause is present such as weak membranes or a patulous os, the membranes may rupture and labour may be started.

Spleen and intestine.

In animals under anæsthesia injections of quinine produce a marked reduction in the volume of the spleen. In pathological enlargements of this organ such as those occurring in malarial fever, administration of quinine causes contraction and reduction in its size. These effects are probably brought about by the contraction of the involuntary muscle tissue in this organ. The intestine may also be contracted.

Fate of quinine in the body.

Quinine is easily absorbed from the mucous membrane, subcutaneous and muscular tissues and it circulates in the blood as quinine base. The length of its stay in the blood is very short. When quinine is given in large and continuous doses by mouth, a concentration in the blood of from 3 to 10 mgm per litre can be obtained. Doses of 2 gm a day must be given to obtain a concentration of 10 mgm per litre in the blood; and when the concentration rises above this, severe symptoms of cinchonism arise. An intravenous injection of 0.8 gm of quinine gives a concentration of 200 mgm per litre, but the drug is rapidly removed from the blood. Of the quinine present in the blood, some is in the blood corpuscles and some in the serum.

Morgenroth's observation showed quinine is preferentially taken up by the red blood corpuscles, and in that position either kills the intra corpuscular parasites or exercises a repellant action on the merozoites which are prevented from penetrating them and therefore perish.

A lage proportion (about 60 per cent) after absorption is deposited in such organs as the liver, gall bladder, kidney, supra-renals, lungs and spleen. In the liver, the quinine molecule is split up and the drug is partly destroyed. The quinine which escapes destruction in the liver and other organs, passes through the circulatory system and is eliminated as quinine base in the urine. The following distribution of quinine in different organs has been given by Ramsden:—Serum 0.1326 mgm., corpuscles 0.005 mgm., liver 14.85 mgm., kidneys 4.025 mgm., supra-renal 7.02 mgm.

Quinine occurs in the lungs in large quantities. Experiments show that after ingestion per os or by injection, guinea-pigs show a much greater proportion of quinine in the lungs than in the liver. This is also the case with optochin and for that reason these compounds were recommended in pneumonia. In cats and dogs, 95 per cent of quinine disappears from the blood in about 5 minutes, after intravenous injection but traces may be found as long as 15 hours afterwards. It is promptly fixed by the lungs, liver, heart, kidneys and brain in higher concentration than the average for the remaining portion of the body; it is given up rapidly so that after 3 hours scarcely any trace of quinine can be found in these organs. It is suggested that fixation by the capillaries leads to more intimate contact between the protozoa affected and the alkaloid. King and Acton (1921) showed that the plasma and the corpuscles contain an equal amount of quinine.

Excretion.

Of the amount taken up by the body, the greater part (60 per cent) is broken up probably by a thermolabile ferment found in the liver; most of the remainder is excreted unchanged in the urine. Small amounts pass out with the bile and traces may be found in the sweat and saliva. Quinine is thus excreted almost entirely by the kidneys. When given by the mouth small quantities occur in the fæces, but this is due to deficient absorption. By the kidneys it is excreted unchanged and if this organ is healthy the process is very rapid, quinine appearing in urine within 20 to 60 minutes of administration by the mouth on an empty stomach. The excretion reaches its maximum in three to five hours, and this maximum rate is maintained for 3 to 4 hours, after which it diminishes. The excretion is complete in sixteen to forty-eight hours. During continued administration of large amounts, elimination keeps pace with the administration, if the daily dose is not more than 30 grains (2.0 gm). About 40 per cent of quinine taken is excreted in the urine, and of this quantity two-thirds is excreted in the first 12 hours, and most of the remaining third within the next 24 hours; after 36 hours only traces are detected.

There is difference of opinion regarding the influence of the form of administration, division of dose, etc. on the rate of excretion of the drug. Soluble salts like quinine bihydrochloride and insoluble salts like quinine sulphate are excreted at the same rate in the urine. The amount of quinine excreted and rate of excretion of the alkaloid are the same whether the drug is given by the mouth, intramuscularly or intravenously. The consensus of opinion is that large spaced doses are most efficacious; that the greatest concentration in the blood is reached in about six hours and the maximum therapeutic effect somewhat later. It has been variously stated that excretion or destruction is increased during fevers. In nephritis and in cardiac decompensation excretion is hindered. Quinine is considered by some authorities to have a well-marked diuretic effect by its dilating effect on the kidney vessels. It is said therefore to be useful in certain forms of œdema and dropsy, especially those due to anæmia and cachexia.

In malaria, the excretion rate is similar to that in health but variations are greater after oral administration. Quinine is excreted in milk (nursing mothers).

5. Therapeutics of Cinchona Alkaloids

Historical and general.

The use of cinchona in the treatment of malaria dates as far back as 1657. The ship surgeons who visited India from 1657 to 1804 used cinchna bark in the treatment of ague. From 1804 to 1847 the cinchona bark treatment of fevers was entirely abandoned in India and treatment by purgatives such as calomel, and venesection was resorted to. In the meantime Pelletier (1820) discovered quinine which then was a mixture of all the alkaloids of cinchona bark. Subsequent to this the use of the bark declined and quinine sulphate gradually took its place. In the meantime other alkaloids such as cinchonine, cinchonidine and quinidine were discovered, and in 1866 the Madras Cinchona Commission was appointed

to test their relative merits in the treatment of malaria. The tests were rough and mostly clinical and the Commission came to the conclusion that quinine was preferable to the other alkaloids. Next MacGilchrist (1914) tested a number of these alkaloids clinically, and he placed them in the following order from point of view of their anti-malarial efficiency:—(1) Hydroquinine hydrochloride, (2) Cinchonine sulphate, (3) Quinine sulphate, (4) Quinidine sulphate, (5) Optochin hydrochloride, (6) Cinchonidine sulphate, (7) Quinoidine. Acton (1920) found dextro-rotatory alkaloids (except cinchonine) more toxic to *P. caudatum* than their laevorotatory isomerides. The corresponding hydro-alkaloids were less toxic. Further, the degree of alkalinity of the environment is of very great importance. The decision as to which alkaloid should be employed rested between hydroquinine, quinine and quinidine. Later experience showed that quinidine has a depressant action on the heart and in some patients untoward effects were produced by it. Cinchonine is a toxic alkaloid and produces irritation of the gastro-intestinal tract and symptoms of cinchonism. Cinchonidine was the least toxic of all and in ten grain doses was quite effective against malaria. A combination of these alkaloids such as that occurring in 'cinchona febrifuge' or tota-quina was just as effective in the treatment of malaria as quinine. Sinton (1924) showed that in malaria there was some evidence of acidosis and therefore he advocated large doses of alkalies along with quinine.

(1) Quinine in Malaria

Quinine and other alkaloids.

Quinine still holds the foremost position in the treatment of malaria, though the antimalarial value of the other alkaloids of cinchona bark is being more and more appreciated. In considering the relative therapeutic value of the different alkaloids it is of great importance to take into account their effect in causing harm or inconvenience to the patient. It may be said in favour of quinine, that it can as a rule be given in sufficient doses and for a sufficiently long period without fear. Cinchonine is more nauseating than quinine and is liable to produce nausea and blurring of vision. Quinidine is also more nauseating and has a powerful depressant action on the heart. Cinchonidine is neither very toxic nor irritating, but it is distinctly the weakest of the four chief alkaloids. Quinine is therefore to be preferred.

Quinine treatment of malaria is no *therapia magna sterilisans.* However large and however frequent the doses, relapses are inevitable. The malaria parasites are not influenced to the same extent at their various stages of development. The youngest forms are the most susceptible and the gametocytes have the strongest power of resistance, they are not noticeably affected in the body by the quinine.

There is still a considerable difference of opinion regarding the methods of administration dosage and duration of treatment. This is largely due to the varying incidence, severity of infection and the resisting power of the several varieties of malaria in different parts of the world. It is therefore very difficult to lay down any hard and fast rules regarding these points, which may be of universal application.

Duration of treatment.

With regard to duration of treatment of malaria with quinine, there are two schools of thought. According to some authorities the relapse rate is not decreased by prolonged treatment with quinine and there is risk in giving a prolonged treatment. According to others a prolonged treatment with 10 to 20 grains of quinine lasting for several weeks is advisable to eradicate the disease. In the author's experience in India, a short course lasting from 5 to 10 days with each paroxysm of malaria have been found to be satisfactory. With the majority of patients in this country prolonged courses are difficult in practice and reduction of relapse rate with such courses is small. We, therefore, advise treatment of every attack of malaria with 5 to 10 days of treatment with cinchona alkaloids.

The following main principles may be laid down in the treatment of malaria with quinine.

1. The alkaloid should be administered immediately after the diagnosis of malaria is assured, irrespective of the stage of the disease and the height of fever.

General principles.

2. Quinine should be given by the mouth when it can be administered and absorbed by that route.

3. The administration of the alkaloid must be continuous over a period of at least 5 to 10 days. Some authorities recommend considerably longer courses but these appear to be unnecessary.

4. Quinine will not act efficiently if the liver is sluggish or congested, or if the gastro-intestinal tract is disordered. To obviate these difficulties a brisk purgative, *e.g.*, 2 to 5 grains of calomel or an ounce of castor oil should be given on the day on which the quinine treatment is begun. Quinine should preferably be given in the form of solution and should be well diluted rather than in a concentrated form. This will aid absorption and will prevent its upsetting the digestive functions. If tablets or pills have to be given they should be crushed and given with plenty of fluid.

5. The administration of quinine should be so timed that it reaches the blood stream at the moment when the latter is at its alkaline tide. The portal blood stream reaches its maximum alkaline tide some 2½ hours after a meal.

6. The alkaloid should preferably be administered with alkalies as these tend to increase the absorbing power of the intestinal mucosa for cinchona alkaloids from the small intestine.

7. In the treatment of all cases of fever it should be remembered that it is wrong to continue giving large doses of quinine for many days if it is not producing any effect. Quinine does good quickly if it is going to be of any use at all. Whether treating ordinary cases of primary attack or of relapse, it is seldom necessary or advantageous to give 20 to 30 grains of quinine daily for more than 5 to 10 days. If the fever does not subside some other factor is responsible for its production and the treatment should be changed.

The two plans for treatment of the disease are:—

(*a*) Short course treatment. It is believed long continued treatment does not prevent relapses and this is the view held by the writer so far as Indian malaria is concerned. Medication for longer periods than 7 to 10 days is not recommended.

(*b*) Prolonged course treatment. After treatment of the attack the quinine is continued in daily doses of 7 to 10 grains for many weeks.

Short course treatment.

The patient should be put to bed before the quinine treatment is started and he should be kept in bed for at least three days if not longer, however mild the attack may be. Fifteen to twenty grain quinine should be given daily, divided into 2 or 3 doses, according to the weight and age of the individual. Some clinicians give the drug every six hours on the assumption that the action of a dose lasts for six hours. As the febrile paroxysm which follows sporulation of the parasites usually begins between 2 a. m. and 12 noon, it is held that doses should be given commencing at 12 midnight. Whatever method is adopted the parasites disappear from the blood and the fever ceases within 24 to 72 hours, but the treatment should be continued for at least five to ten days after the fever subsides.

Sometimes, quinine cannot be given by the mouth owing to persistent vomiting, especially in pernicious cases. In such cases the best method of administration is by the intramuscular route. Ten grains given in this way produce cessation of vomiting and other acute symptoms within a few hours, and disappearance of parasites in 18 hours. Quinine can then be given by the mouth.

Prolonged course treatment.

The following plans have been suggested for prolonged treatment which is continued for weeks:—(*a*) 10 grains of quinine once daily, (*b*) 30 grains of quinine on each of two consecutive days, *e.g.*, Saturday and Sunday each week, the amount being preferably taken in 4 separate doses, (*c*) 30 grains of quinine once a week, *e.g.*, Sunday, in four doses (*d*) 15 grains on each of two consecutive days, *e.g.*, Saturday and Sunday each week, (*e*) 15 grains of quinine only one day each week, *e.g.*, Sunday. The amount in the last two plans is taken in one dose and is suitable for mild attacks. The daily dose plan would appear to be the best as it is difficult to state on which day the parasites will appear in the circulation in sufficient numbers to produce a paroxysm. Ten grains given every day will probably be sufficient to destroy the parasites in the circulation. Larger doses given at longer interval may compensate, but the chances of paroxysms recurring are greater. Such doses may not prevent reinfection of the patient, and whenever it is possible these should be prevented by other means. James (1922) laid stress on the time of taking quinine and considers that success of treatment depends on this. The dose, he considers, must be taken 2 to 3 hours before the onset of the febrile paroxysms. If a relapse occurs the whole course including the 5 days treatment of the attack should be repeated. The removal of patients from an endemic area to a better locality is often helpful. Patients suffering from malaria who do not do well in tropical climates immediately start improving when removed to a better climate. It should be remembered that removal to a very cold climate may light up an infection and bring about a severe relapse.

A number of standard treatments of malaria with quinine have been suggested to suit various localities.

(1) Ronald Ross (1921) advises during the first two weeks, 15 grains of the hydrochloride or sulphate of quinine daily; then 10 grains daily for six days in the week for eight weeks making a total of 690 grains. Benign tertian infections yield rapidly to this treatment as regards the febrile attacks, but are more difficult to cure in the sense of preventing further relapses than the malignant tertian type. Twentyfour thousand cases treated by the Ministry of Pensions were cured by this plan.

(2) The United States Malaria Commission (1921) recommends 30 grains (2.0 gm.) quinine sulphate daily in 3 doses while the symptoms last and for 4 days after; this is followed by 10 grains every night before retiring for 8 weeks. In Palestine this course was insufficient to cure chronic cases. The Jamaica Tropical Diseases Conference did not consider it advisable to recommend this method for universal application.

(3) In South Africa, Pratt-Johnson and Gilchrist (1921) advise 10 grains of quinine 3 times a day for three weeks, then 10 grains twice a day for one month followed by 10 grains once a day for two months. They found the relapse interval was 14 days in benign tertian and 12 days in malignant tertian cases; the crescents disappeared, on an average, in 15 days with 25 grains of quinine a day.

(4) Panama Canal Zone Standard course (1925) is more drastic. The patient is given a dose of 2 to 3 grains of calomel followed by a dose of Epsom salts before the treatment is started. Fifteen grains of quinine are given three times a day until the temperature settles down and is normal for 5 to 6 days. Then ten grains are given three times a day for 10 to 14 days. This course cured the vast majority of malignant tertian infections.

(i) Synergist of quinine

Combined alkali and Quinine treatment.

Acton (1921) first recommended the association of alkalies with quinine, with the idea of reinforcing its action. He found that the toxicity of this alkaloid on paramœcium and bacteria was increased when the hydrogen-ion concentration of the medium was on the alkaline side. Sinton and Lal (1924) gave large doses of alkali to patients to produce such a condition in the body. They found by means of Sallard's clinical test of giving by the mouth, 4 gm of sodium bicarbonate well diluted, then 2 gm an hour later, followed by 2 gm every half an

hour and testing the urine with litmus till it becomes alkaline, that there was acidosis in malaria. Acton and Chopra (1925) showed that the administration of alkalies before quinine, increases its rate of diffusion through the mucous membrane of the intestines. By a previous administration of alkali the concentration of quinine in the mesenteric vessels can be greatly increased.

Alkaline mixture A.

Sodium bicarbonate	60 grains
Sodium citrate	40 „
Water	1 ounce

Quinine mixture Q.

Quinine sulphate	10 grains
Citric acid	30 „
(Or Dilute Sulphuric Acid)	30 min
Water	1 ounce

All cases are given calomel and magnesium sulphate before the treatment; for the first day of treatment 3 doses of A are given at 7-30, 9-30 and 11-30 a.m.; half an hour after the last dose one dose of Q is given. At 6 p.m. a further dose of A is given followed 15 to 20 minutes after by one of Q. For the next 4 days one dose of A is given three times a day at 7-30, 11-30 and 6-0 p.m., followed on each occasion by a dose of Q. For the remaining 2 days one ounce of A is given morning and evening followed 15 to 30 minutes after by a dose of Q. Controls were given mixture Q only but no alkali. The total quantity of quinine given in 7 days was 180 grains and the criterion of cure was non-appearance of parasitic relapse during 8 weekly examinations.

Sinton (1930) expressed the opinion that a one-week course suffices in the majority of cases. Though a prolonged course of treatment gives a slightly higher rate of permanent cures, the difference is not sufficient to warrant the extra time or expense. He also recommends plasmoquin 0.015 gm daily throughout the course, to be given after food. If a relapse occurs the whole course should be repeated for one week, and continued for a second week, with two doses instead of three.

If the alkaline mixture produces gastric disturbances, 2 drachms of sodium citrate in 2 ounces of water is taken slowly. Alkali treatment can be combined with cinchona febrifuge. In severe cases a dose of alkaline mixture followed by 1 ounce of quinine mixture 15 to 30 minutes later, may be given at once without waiting for the purgative, but in these cases alkaline treatment should be continued till the urine becomes alkaline. In severe cases of malignant tertian malaria, the amount of sodium bicarbonate may be increased to 90 grains dissolved in two ounces of water.

Advantages.

(1) With this treatment in Sinton's series, 28 per cent, of benign tertian relapsed as compared with 40 per cent without alkali treatment; whilst in case of malignant tertian the relapse rate was 15 per cent as compared with 79 per cent. (2) Albuminuria is not seen if alkalies are combined with quinine. (3) In case when quinine cannot be given by mouth due to attendant vomiting—administration of alkaline mixture makes this possible.

Quinine and alkali-producing substances (citric acid is converted into an alkali in the gut) can be prescribed in one mixture especially for outpatients in which it is not possible to carry out Sinton's treatment.

Quinine sulphate	10 grains
Citric acid	30 „
Magnesium sulphate	30 „
Chloroform water	1 ounce

This mixture is given 3 times a day 2½ hours after food for one week. The dose is then reduced to 1 ounce a day for further two weeks. Citric acid is converted in the gut into carbonate and this acts as an alkali.

After treatment

After-treatment is an important consideration in malarial patients treated with cinchona alkaloids. At the end of a course the patient is anæmic and run-down and a combination of iron, arsenic and strychnine in small doses is often beneficial in building up the resistance of the patient and in the destruction of the remaining parasites not destroyed by quinine treatment. In some cases it has been found that quinine apparently produces little effect and the patients relapse. In such cases a dose of Novarsenobenzol, before quinine treatment, is helpful to overcome infection but this should be given with caution.

Provocative agents.

Believing that the appearance of parasites in the blood in chronic cases of malaria renders them more liable to the action of quinine, certain substances have been used as activating agents to produce malarial relapses for securing the reappearance of asexual parasites in the peripheral blood. For this liquid extract of ergot 30 minims, five doses in all, or thyroid extract have been suggested. Subcutaneous or intramuscular injections of normal horse serum, milk and strychnine nitrate 2 to 3 mgm are used; application of X-Rays, ultra violet rays, cold douches, ice, etc., to the abdomen have also been suggested. These methods are not very reliable and the effects produced may not be immediate; several days may elapse before the parasites appear in the peripheral blood. Adrenalin, 1.0 mgm (1 ccm of 1 in 1,000) intramuscularly, gives the highest percentage of positive results by producing a marked but temporary contraction of the spleen volume. Intravenous injections of neosalvarsan in doses of 0.3 to 0.4 gm and ureastibamine or neostibosan in doses of 0.25 gm are also effective. These however are not without danger.

Provocative agents.

Ascoli's treatment.—This method which was used in Italy, consists in giving intravenous injections of adrenaline in increasing doses, starting with 1/100 mgm then 1/90 and so on till a dose of 1/10 mgm is attained. The size of spleen is reduced and the efficiency of quinine in resistant cases is said to be increased.

(ii) Dosage

Dosage and time of Administration.

In non-malarious countries very small doses such as 2 or 3 grains are given. In tropical countries, the tendency is rather towards giving too large doses. It has been said that less than 20 grains of quinine in a day in an adult has no curative effect in malaria. This is not borne out by James' observations with induced malaria, who found that a few grains a day kept the patients suffering from induced benign tertian infections free from paroxysms for months. With such small doses however relapses occurred after long periods. Generally 20 to 30 grains by the mouth in 24 hours according to the weight of the individual, should be regarded as the maximum dose. Such doses always control malarial fever and when they do not, either the drug is not being absorbed or the diagnosis is wrong.

A maximum dose of ten grains of the sulphate or hydrochloride by the mouth, two or three times a day for an adult, given for one week is quite sufficient to cure 70 per cent of fresh malarial infections. Twenty grains a day are sufficient in most cases and in women and weak individuals, fifteen grains will

suffice. Larger doses, as a rule, have no advantage. To be effective, however, quinine must be in the body in sufficient concentration when parasites are most abundant, *i. e.*, at the beginning of an attack, before and during the shivering stage and immediately afterwards. The old practice of giving an adequate dose four to six hours before another attack was sound, but the attacks do not always occur at calculated hour. It is, therefore, advisable to give the drug in several doses distributed over a number of days. It is suggested by some workers that after prolonged and continuous use of quinine the action of quinine may become less effective.

In relapsing cases 5 to 7½ grains twice daily for one month in the case of malignant tertian infections and three months in the case of benign tertian and quartan infections are recommended by some. Doses larger than 20 to 30 grains daily lead to toxic symptoms, especially in weak individuals and are an absolute waste of the drug. The consensus of opinion at present is that a 7 to 10 days' course of treatment with 20 to 30 grains of quinine according to the weight of the patient is sufficient in all forms of malarial fevers. If there is a relapse, the whole course should be repeated.

Liver damage.

The liver of rabbits is damaged by giving repeated intravenous injections of quinine, the cells of the parenchyma showing degeneration and fatty infiltration; the same may happen in man with large doses of quinine. Amounts larger than 30 grains a day are, therefore, by no means devoid of danger. Nierenstein (1920) stated that over 30 grains of quinine per diem are liable to injure the kidneys and cause albuminuria. It should not be forgotten however that acute and sub-acute parenchymatous nephritis may occur as an accompaniment of malaria and is readily benefited by quinine treatment. Massive doses also depress the cardio-vascular system and retard development of natural immunity on which the cure of the disease depends (McCarrison and Cornwall).

Maximum dose.

Quinine should not therefore be given in doses larger than 30 grains daily; even such small doses as 5 grains two or three times a day given under rigid supervision are sufficient to cure an attack in persons weighing 100 pounds, but such small doses are not recommended for routine administration. It is preferable to give the larger doses already suggested, *i.e.*, 10 grains two or three times a day.

(iii) Causes of failure of quinine therapy

If quinine is properly administered and absorbed it is bound to cure a large majority of the patients. The causes of failure may be:—

(1) Use of insufficient doses. Hospital mixtures supposed to contain 10 grains to an ounce have been found to contain 1 to 2 grains in India. (2) Adulteration of quinine. Quinine adulterated with lime, starch and other inert substances to the extent of 60 or 70 per cent has not infrequently been met with in India; this is also the case with tablets. (3) Faulty preparation. Tablets may be prepared with unsuitable media and may have a coating quite impervious to water. (4) Some of the patients may not be taking the quinine prescribed for them owing to their dislike or prejudice against the drug, and they may be deceiving the physician. (5) Non-absorption from the gastro-intestinal tract due to gastro-intestinal disturbances produced by the drug itself or complicating diseases such as chronic dysentery, ankylostomiasis, enteric, etc. (6) Low individual resistance. It is well known that some persons catch infection quickly and get rid of it with difficulty. (7) Resistance of particular strain. Some local strains are known to be more readily influenced by therapeutic agents than others and these, therefore, require more intensive treatment.

When quinine is being administered the physician should make sure:—

1. That quinine prescriptions are in full strength. There is evidence that mixtures in India are sometimes much below strength.

2. That the patient swallows and retains every dose prescribed and that he does not omit to take a single dose of the standard course given. Many causes may lead to omission, *e. g.*, vomiting may occur or the patient may wilfully reject a dose.

3. That the patient is absorbing quinine or any other alkaloids given. This may be ascertained by testing the urine. Take 5 ccm of urine, boil, and filter the albumin if present and add a few drops of Tanret Mayer reagent. A precipitate is formed if the alkaloid is being excreted in the urine. The drug should be given in hospitals by a responsible individual who sees that the mixture is swallowed. One or more of these factors may account for the failure of quinine to cure malaria. Intramuscular treatment may succeed in certain conditions when quinine by the mouth has failed for obvious reasons.

(iv) Relapses

Relapse rate.

Relapses occur frequently in spite of proper treatment with quinine. Administration of iodides, adrenaline, sera or vaccines, exposure to ultra-violet rays, excessive heat or cold, bring about a relapse and should be avoided. In addition to the selective action of quinine on the type of parasite involved, the chronicity or acuteness of the infection is an important factor. Sinton and Bird (1929) found that quinine cured 76 per cent of primary infections, but in chronic cases it cured only 32 per cent the balance all relapsed. Chronic benign tertian infections are more difficult to eradicate. These results were corroborated by treatment of induced benign tertian malaria for the cure of mental diseases, which was easily amenable to quinine; the relapse rate was only 1 to 3 per cent with 30 grain daily doses for three days. The reason why primary infections of benign tertian are more readily cured than chronic infections is not understood; some observers have stated that the parasites become more resistant.

Inherent resistance of any strains of parasites is denied and only an acquired resistance is accepted by many authorities. This may occur partly through unsuitable treatment and partly through continuous hyper-infection during quinine medication. Resistance may also be symptomatic of causative factors, such as fatigue, malnutrition, injuries and the like, exercising a provocative influence on the patients system. Relapses are to be expected sooner or later and especially in benign tertian and quartan infection; though malignant tertian yields more rapidly to a single treatment. The chances of cure are enhanced by using other available remedies. When for example a relapse occurs after continuous quinine therapy, it may be treated with atebrine, and if it occurs after atebrin it may be treated with quinine (Nocht & Mayer, Malaria).

In the writers' opinion every relapse, whatever be the species involved, should be treated immediately with full doses just as if it were the primary attack.

Quinine Pyrexia.

So-called Quinine Fever. By this term is meant pyrexial phenomena without any or only a few malarial parasites occurring in patients taking small or medium doses of quinine. This is not produced by the alkaloid, as was supposed but is probably due to administration of insufficient quantities of quinine which are unable to control the infection. If the quantity of the drug is increased and adequate doses are given for 3 or 4 days at proper times, the fever disappears. Chopra and Das Gupta (1933) however observed a distinct increase in the parasites in the blood after intravenous injections of quinine in man and monkeys. The temperature often rises after quinine. This may occur alone or with other symptoms (paradoxical Quinine fever). These symptoms may develop with or without injection.

(2) Routes of Administration

Quinine may be given by the mouth, subcutaneously, intramuscularly, intravenously or per rectum. By all these methods it is absorbed into the circulation.

Oral route

Oral administration. On account of the convenience of administration, the oral method is the one of choice. It may be given as a powder, in solution, in cachets or in the form of pills or tablets. When given in solution it is more readily absorbed than in any other form and therefore, this is usually employed. The usual mixture employed in hospitals is 5 to 10 grains of quinine sulphate, with 5 to 10 minims of dilute sulphuric acid in an ounce of water. Some prefer the bisulphate or hydrochloride, but these are more expensive. There is no evidence to show that the soluble salts are any more effective than the insoluble sulphates, though the absorption of the latter is somewhat slower. Tablets are more convenient and are less unpleasant than the solution, but they may not be absorbed.

Even quinine in powder form is not recommended by many authorities. The powder and tablets take a longer time to absorb than the solution, but whether given in solution or in solid form, the alkaloid is absorbed into the circulation quite readily. The form of administration adopted should be such as to suit the individual patient. Uncoated tablets whether of the sulphate or of the soluble salts, if fresh, are readily dissolved and are absorbed. If there is any doubt, they should be crushed and then administered. Some physicians prefer to give quinine in solution during the stage of active fever and to carry on with tablets during convalescence.

Clinical condition the best guide.

The clinical condition of the patient is the best guide in deciding what method should be adopted. The condition of the tongue is an excellent indication of the state of the gastric mucosa. In ordinary attacks the tongue is moist and slightly furred, and under these circumstances the alkaloids are absorbed very well from the gut. If the tongue is dry, red and cracked, quinine is absorbed badly, and in such cases other means should be adopted. Quinine is generally absorbed readily from the gut; even in cases of malaria complicated with dysentery, quinine has been shown to be absorbed. If nausea and vomiting are present or there is the bilious vomiting of malignant tertian infection, it is useless to give quinine by the mouth. Vomiting may have to be stopped by adrenaline in doses of 0.5 to 1 ccm of a 1 in 1,000 solution, by the mouth. Provided it is retained, quinine by the mouth is as efficacious as by injection. Experiments have shown that in solution it is absorbed with as great rapidity and appears in the urine almost as rapidly as when given by the parenteral route. It is, therefore, the safest and the best method except when its effects on the alimentary canal are not desirable.

On account of its bitter taste quinine is unpleasant to take. This disagreeable taste can be disguised by adding glycerine one dram, or syrup of orange one dram, to each dose. By mixing quinine with milk, its taste can be disguised to some extent. Quinine powder may be mixed with a tea-spoonful of milk powder. In children euquinine may be given. Another way to disguise the taste is to give quinine in an effervescent mixture.

Frequency of administration.

The rapid absorption of the alkaloid and its quick disappearance from the blood, indicate the importance of ascertaining for each case, the time at which the drug should be administered, in order that it may destroy the vulnerable stages of the parasites. In clinical work James recommends that two facts should be remembered:—(1) sporulation begins about 2 hours before symptoms manifest themselves, (2) that the effect of a dose of quinine by the mouth begins within half an hour after its ingestion and lasts for more than six hours. He stresses the advantages of giving the drug 2 or 3 hours before the attack is expected.

When more than one group of parasites are present, as in malignant tertian infections, sporulation occurs at irregular intervals and the plan of giving the drug at short intervals is the best. Others hold that quinine should be started when the schizonts are very young, as growth then ceases and degeneration ensues; if it is given when the schizonts are half grown, some are killed, some degenerate, while others carry on to sporulation. Quinine given during sporulation does not stop the paroxysms.

Experience shows that best results are obtained when these alkaloids are given 2 or 3 times daily in adequate doses. As the concentration of the alkaloid rapidly falls between 4 to 8 hours after ingestion it is considered advisable to administer the doses at intervals not longer than 6 hours.

Quinine during fever.

The older writers recommended that quinine should not be given during fever, and even now this idea prevails in the mind of the public. There is no basis whatever for this belief. Even high fever is not a contra-indication for its administration. In malaria, quinine should be given regularly and continuously irrespective of temperature. Quinine has been shown to be just as effective in the febrile period as in the non-febrile period. It has also been said that quinine should not be prescribed with free organic acids as quinotoxin is formed, which is very poisonous. That is not true because quinotoxin is not a toxic substance.

Rectal route.

Rectal administration. Quinine is much too irritating and in concentrated solutions may actually produce necrosis of the mucous membrane. This method is therefore not satisfactory and is rarely employed. Dysentery-like symptoms were produced with 30 grains of quinine in 2 to 4 ounces of water. The absorption of quinine by this route is uncertain and poor, and about three times the normal dose is required. The drug is seldom retained long on account of the pain and tenesmus which is produced. If diarrhœa or irritation of the bowel is present the solution is rejected. When it has to be given by this route it is advisable to give it in the strength of 10 to 15 grains in 4 to 5 ounces of warm normal saline mixed with 10 minims of tincture opii. The fluid is slowly injected with a rubber catheter. Fletcher (1924) has shown that absorption of quinine by the rectal route, as judged by testing the urine with Meyer's reagent, is poor, irregular and unreliable. He advises that it should not be given by this route if any other method is practicable.

Subcutaneous injection.

Subcutaneous route. This method has been used though some physicians condemn it. Subcutaneous injections are painful and liable to produce necrosis; they are said to be followed by extensive and dangerous abscess formation and even tetanus. On the other hand it is said by some to be perfectly harmless and ensures rapid absorption of the drug. The manipulation is simple and can be safely entrusted to an assistant. In Russia subcutaneous injections of 0.5 to 1.0 gm. of quinine hydrochloride are given in all cases, in combination with antipyrin and iodine. Rarely, subcutaneous tumours have formed after injections of quinine.

Intracutaneous injections of 0.25 to 0.5 per cent solutions of quinine bihydrochloride have been suggested in the treatment of chronic malaria. Very small doses of quinine given by this method are said to be effective on account of antibody formation but this is doubtful.

Intramuscular injection.

Intramuscular route. This method of administering quinine is simple, but it should be carried out with strict aseptic precautions and the skin should be throughly sterilised with tincture of iodine. The usual indications for administration by this route are, bilious remittent fever, persistent vomiting, drowsiness, or mental affections; also when the quantity of the alkaloid available is small as during World War II.

The syringe and the needles must be kept in absolute alcohol which is washed out with ether before the injection or they should be thoroughly boiled. It is advisable to use ampoules or sterules, but if these are not available the solution prepared for injection should be carefully sterilised. Bihydrochloride of quinine, preferably dissolved in distilled water, is employed and it should be

thoroughly boiled. Sergents advise that the strength of the solution should not exceed 3 per cent but injections of dilutions as weak as 1 in 150 are known to have caused extensive necrosis of muscle and nerve tissue. If bihydrochloride of quinine is painful, solutions of urethane quinine or quinine and urea have been recommended. It is preferable to use dilute solutions, *i.e.*, 7½ to 10 grains of the hydrochloride or bihydrochloride dissolved in 3 or 4 ccm of 0.85 per cent saline rather than in 1 to 2 ccm, which is usually done. More than two injections of 0.5 gm of quinine in a day should not be given. After injection the part should be massaged and collodion applied. The situations recommended for giving the injections are:—

Selection of site.

(1) The gluteus maximus avoiding the line of the great sciatic nerve. This is the best situation, the spot selected being about 2 inches below the middle of the crest of the ileum. The needle should be introduced perpendicularly and if the point strikes the bone it should be withdrawn about a quarter of an inch and the quinine injected into the muscle. Care should be taken not to inject any into the subcutaneous tissue, as this will cause pain, stiffness and inflammation and very frequently an abscess.

(2) The cellular and muscular tissue below and external to the apex or angle of the scapula. The advantages of this site are that the injections are painless, there is no danger of damaging important nerves, the operation is not visible to the patient and the solution is readily absorbed.

(3) The vastus externus about its middle on the outer side of the thigh.

(4) The deltoid muscle avoiding the line of the musculospiral nerve. The best place is 2 inches below the acromion process.

The injections should not be given in the same place every day as this may produce necrosis and abscess. When the neighbourhood of a previously injected site is selected, inject in the circumference of a circle keeping at least half an inch away from the previous puncture mark. It is advisable to stop the injections and administer quinine by the oral route as soon as the paroxysms have been controlled.

The most suitable preparations of quinine for intramuscular injection are the hydrochloride or bihydrochloride because of their ready solubility. These, however, form an acid solution (pH 3.5) and are liable to produce pain. This can be mitigated by combining urethane five grain with ten grains of the hydrochloride in 2 ccm of water.

Effectiveness of intramuscular route.

Considerable differences of opinion prevail with regard to the advisability and efficacy of intramuscular injections of quinine. Some authorities maintain they should never be employed, while others consider them as invaluable in severe cases, especially the dangerous subtertian and also benign tertian infections which do not respond to oral administration.

According to Fletcher (1924), quinine given intramuscularly causes serious mutilation. The drug by this route is said not to be absorbed so quickly as when given by the mouth, nor is it more potent in preventing relapses. It produces immediate necrosis of the tissues into which it is injected; the dead muscle is usually not absorbed for a few weeks and may remain as a source of disease, forming an excellent nidus for the growth of bacteria from the blood stream. Cases of severe mutilation in the form of paralysis from injection near a nerve, or contraction of muscles from severe suppuration are frequently seen. Injection in the neighbourhood of a large nerve such as the sciatic, produces excruciating pain lasting for days; paralysis of muscles and contractures may occur. It is alleged that quinine given intramuscularly has certain advantages over the oral route and briefly stated these are:

(1) Its action is said to be more rapid and more vigorous. But it has been found that with intramuscular injections quinine appears in the urine (Meyer's test) on an average in 60 minutes, while if taken by the mouth in the form of a solution it appears in about 30 minutes. The action therefore cannot be more rapid.

(2) The effect of intramuscular injection is said to be more prolonged. It is believed in some quarters that quinine given by intramuscular route acts as a kind of reservoir and in this way a steady concentration of quinine is maintained in the blood. The origin for this belief is not known. It is found by actually testing the urine with Meyer's reagent that by whichever route it is given, quinine disappears from the urine in about 46 hours. Experiments on guinea-pigs show that a large amount of quinine is absorbed after injection and only a small fraction of the total quantity injected can be found at the site of injection.

(3) It is said to have a greater power of preventing relapses. Rennie and Acton (1921) found in a malaria depot in the Himalayas (Dagshahi) that the percentage of relapses was the same whether the quinine was injected or given by the mouth.

(4) The therapeutic value of quinine given in this way is said to be greater. A number of medical men believe in the superior efficacy of intramuscular injections, on clinical grounds. Careful experiments have shown that there is little difference between the oral and intramuscular methods; if anything, the advantage is with the oral method. The reason for this belief probably is that by the oral route, administration of quinine has to be left to patients, who may or may not take the full dose of this unpleasant drug. Intramuscular injections are given by the physician himself and it is certain that a known quantity of quinine is introduced into the system. Besides it is not infrequently found that a mixture supposed to have ten grains of quinine has one or two so the only advantage of intramuscular injection of Quinine is that the administration of the drug is assured.

Necrosis of tissues at the site.

The fact that necrosis of tissues is a constant accompaniment of intramuscular injections is not sufficiently appreciated by practitioners. Experiments by Acton and Chopra (1923) on rabbits and guinea-pigs showed that 24 hours after injection of bihydrochloride of quinine and other alkaloids (quinine, cinchonine and cinchonidine) in the concentrations in which they are employed clinically, they produced very extensive œdema of the subcutaneous tissues and muscles round the site of injection; hyperæmia of vessels was very marked and hæmorrhages were present in the substance of the muscles. The bases of these alkaloids were found precipitated in the tissues, and muscles and fascia round the site of injection were friable and necrosed. In one case where the injection was given near the sciatic nerve the sheath showed signs of extensive damage. The damage to the tissues was marked on the 3rd day, but by the 6th day œdema and hæmorrhage had disappeared completely, while necrosis was still visible on the 10th day. Manson Bahr showed that continuous injections at the same site gave rise to patches of necrotic tissue about the size of an almond, but that there was no indication of pus formation. In weak, anæmic and debilitated subjects such dangers may exist. Fibrous tumours have occasionally been formed by irritation produced by quinine.

Occurance of tetanus.

Cases of tetanus have occurred after quinine injections, but these are generally due to faulty technique. The necrosis of muscle caused by injection of quinine forms a suitable nidus for the development of tetanus spores which are taken up by the leucocytes from the alimentary canal and deposited there. Recent work of Gye and Cramer on kataphylaxis has shown that such a possibility does exist. It has been shown that tetanus spores washed free of toxins and injected into an animal do not produce tetanus. If after some months in this animal, quinine is injected, tetanus develops at the site of injection of the alkaloid. This is due to the rupture of local defence mechanism. Quinine produces necrosis of tissues and this hinders the oxidation processes in the cells, the anaerobic conditions thus set up allow the spores to develop.

Conclusion.

It will be seen, therefore, that intramuscular injections of quinine should not be given lightly or without most rigid attention to efficient aseptic precautions, not forgetting that tetanus spores are not killed even at 100°C, for a certain length of time. It is not justifiable to give quinine by this route in the treatment of ordinary cases of malarial fever when the patient can take the drug by the mouth. It should be reserved for severe cases of malignant tertian malaria with gastrointestinal symptoms, where quinine cannot be given by the mouth, or in young children and fat patients where intravenous injections cannot be given because the veins are difficult to find. It is true that hundreds of injections may be given without untoward effects, but the very next one may lead to

disaster. Necrosis of the tissues is often a slow process and the results may not be apparent until weeks or months after the injections. Intramuscular injections do not produce cinchonism on account of the slow absorption of quinine.

Briefly stated, the dangers of intramuscular injections are (1) tetanus, (2) necrosis and formation of an aseptic abscess and (3) injury to nerves.

Intravenous route.

Intravenous method. The indications for intravenous administration of quinine are firstly, appearance of a very large number of parasites in the blood such as a dozen or more in a single field and secondly, pernicious malaria with nervous or mental symptoms such as drowsiness, aphasia and nervous twitchings.

Dosage.

It is not advisable to give more than 0.5 gm of quinine in Indian patients by this route at one time. Injections should be given slowly,otherwise collapse may result. Small amounts of quinine, even as low as 0.3 gm, are sufficient to produce immediate favourable results; large doses, *e.g.,* 1.0 gm do not appear to display any greater immediate effects. In addition to intravenous injections, 0.5 to 1.0 gm may be given intramuscularly. In the large majority of cases, temperature returns to normal on the second day and sometimes, though less frequently, on the third day. A sharp rise of temperature may occur after the injection probably due to the large number of parasites destroyed.

Two methods.

Two methods for giving intravenous injection of quinine are recommended:—

(1) By giving it in 4 to 6 ounces (100-150 ccm) of (0.9 per cent) saline. The apparatus for injection consists of a tall glass funnel one inch in diameter capable of holding 8 to 10 ounces of saline. To this 3 feet of rubber tubing is attached and at the end of this a sharp needle is fitted, such as that used for salvarsan injection. A thermometer for taking the temperature is also required. For injection, 7½ to 10 grains of quinine bihydrochloride are dissolved in 8 ounces of normal saline, the temperature of the fluid being 110°F., to allow for cooling during its course through the apparatus. Large quantities of saline used for dissolving quinine may however produce dilatation of the right heart, especially if given too rapidly and collapse or even death may result. On the other hand, if the injection is too concentrated it may produce inhibition of the heart's action, directly or through the central nervous system. Extensive experience has proved that the use of more than 100 ccm fluid for each case, as advised by some, is unnecessary and not devoid of danger.

(2) Injections may be given by means of a 10 to 20 ccm syringe, 7½ to 10 grains of the salt being dissolved in 10 to 20 ccm of sterile warm normal saline. Some clinicians dissolve 10 to 15 grains of the bihydrochloride in 5 ccm of normal saline, heat it to boiling point and draw up into a syringe and inject. One of the superficial veins at the bend of the elbow is selected for the injection and a clean puncture should be made with a sharp needle. The fluid is slowly injected, the needle pointing towards the shoulder; several minutes are taken to complete the injection. The most rigid antiseptic precautions should be observed.

Indications.

The intravenous route is especially recommended in serious and dangerous malarial infections, *e.g.,* cerebral malaria, where the effect of the drug has to be produced in a very short time and a few hours' delay is likely to be fatal. Many cases are lost owing to the hesitation on the part of practitioners in using this simple, quick and efficient method of controlling symptoms in grave cases. In chronic malaria when administration by the mouth fails to be effective, a few intravenous injections may free the patient from parasites. One dose of 10 to 15 grains is usually sufficient to stop the fever and to cause the disappearance of most of the parasites within 18 hours. The injection is repeated, if necessary, in 3 or 4 hours if by that time oral administration is not feasible. These injections should be given in such a way that they reach the heart in low concentration. Experiments on rabbits show that there is considerable difference in the minimal lethal dose of quinine, according as to whether it is given well diluted and slowly injected, or given in concentrated form and injected rapidly. The minimum lethal dose by the latter method is much smaller.

Caution.

The pulse should be carefully watched. If the injection is given too rapidly the pulse may become very feeble and alarming symptoms set in. Rigors sometimes appear within a few hours after injection; this is due to acidity of the salts, but it soon passes off. Some authorities advise the addition of a few drops of adrenalin solution to counteract the fall of blood pressure, but it seems to be unnecessary except in very severe cases of pernicious type. In some patients alarming symptoms appear in spite of all precautions. If the pulse becomes feeble give strophanthin or digitalin. If the injections are given slowly in a recumbent posture, the dangers of cardiac syncope are considerably reduced.

Disadvantages.

It has been demonstrated that large doses of ordinary quinine salts, *e.g.*, quinine hydrochloride, when given intravenously, are dangerous to life, the respiratory centre being more gravely affected than the cardiac centres. Quinine acid hydrobromide in 7 to 10 grain doses is less toxic to the respiratory centre than the acid hydrochloride and is preferred. Injections should be controlled by blood pressure observations especially in weak patients. It has been found, in experimental animals, that if intravenous injections are given continuously, circumscribed areas of necrosis appear in the adrenal cortex. Though the action of quinine injected direct into the blood stream is quicker than that of quinine given by the mouth or by the intramuscular method, it has no greater effect in destroying the gametocytes or eradicating the parasites from the body and so preventing relapses. Its action is more fleeting than when the drug is given by any other route, and for this reason, unless the administration of a dose is timed correctly in relation to the parasites, it is wise to supplement it as soon as possible by doses given orally. There is considerable evidence to show that quinine has little effect upon parasites contained in the red blood cells which are lying more or less stagnant in the small capillaries of the internal organs. It is chiefly effective against parasites which are free in the circulation, especially when carried to the peripheral parts of the body.

Dangers.

Acton and Knowles (1924) pointed out that the dangers of intravenous injections have been exaggerated. Phlebitis and thrombosis very rarely occur. Air embolism is a bogey; if proper care is taken 7½ to 10 grains of acid hydrobromide in 15 to 20 ccm of sterile water is quite safe and can be conveniently given by a syringe. Larger quantities such as 15 grains in 5 ccm of saline are not recommended as being too concentrated. It should be remembered that while the solubility of quinine hydrochloride is 1 in 35 of distilled water, it dissolves in 110 parts of Ringer's solution. About 90 per cent of quinine injected disappears from the blood in a minute, and during that time it circulates in the blood in maximum concentration and has a destructive action on those parasites which have not reached the safe areas within the erythrocytes.

Routine Treatment.

Cantlie and Moubarek (1924) in the Sudan treated a large number of cases of malaria by intravenous injections of quinine. They gave 9 grains of bihydrochloride, every day till the temperature became normal, as well as ten grain doses of bisulphate by the mouth. The injections occupied 30 to 45 seconds, the pulse was accelerated 5—17 beats per minute for not more than 3 minutes. Tingling in the mouth, ringing in the ears, sometimes constriction of the chest, rarely vomiting and on two occasions fainting occurred. Their observations showed that the action was more powerful and thereby the stay of the patient in hospital was cut short, but the relapse rate was 42.5 per cent. In another series of 1,103 cases there was a relapse rate of 77 per cent after 6 to 8 injections in each case. A dose of 0.5 gm of quinine given intravenously is as effective as 1.5 gm by the mouth. If given on a fever-free day in benign tertian malaria, the paroxysm of the next day was cut short in 85 per cent of cases against 64 per cent with oral administration. Injections are also said to be effective in chronic malaria when administration by the mouth is not effective. Thrombosis of the vein often prevented the continued use of this method.

Conclusions.

Intravenous injections of quinine should therefore be reserved for cases of special urgency such as cerebral malaria with delirium and coma, or malarial hyperpyrexia or malaria of pernicious type with persistent vomiting. They should

be given without hesitaton in such cases, and as soon as the condition of the patient allows it, oral administration should be started.

Cordes (1928) treated 14 cases of cerebral malaria with 7½ grains (0.5 gm) of quinine in 10 ccm of water and saved all of them. In such cases puncture of the cisterna magna at the base of the brain and removal of 50 ccm of cerebro-spinal fluid is probably preferable to lumbar puncture. Cases with as many as 40 per cent of the corpuscles parasitised, have been saved by injection of 15 grains of quinine daily for three days. Attention should be paid to the following points when giving intravenous injections:

Cautions.

(1) As they produce a fall of blood pressure and affect the respiratory centre, they must be given with the patient in the recumbent posture. (2) The injection must be a clean one into the lumen of the vein, otherwise it will produce necrosis and complications. If the needle misses the vein or slips out, stop the injection at once and before withdrawing the needle, suck back into the syringe all the solution that may have escaped into the tissues round the vein. (3) The injection must be given very slowly, at least 20 to 30 seconds being taken for the injection of each ccm. (4) The solution should be freshly prepared.

The injections are followed by a transient feeling of dizziness lasting a few seconds while quinine can be tasted in the mouth before the injection is completed. It should be remembered that while intravenous quinine therapy is a useful addition in the treatment of critical attacks of malaria, it is useless in the prevention of relapses, and fails to rid the patient of parasites completely.

Intravenous administration of quinine should be given with caution when there is albuminuria, severe jaundice, organic disease of the heart, marked anæmia or debility and idiosyncracy to the drug.

Routes to be used.

Summary. The best method of giving quinine is by the mouth and this is borne out by hæmatological as well as other evidences. It is best given in solution but owing to its very bitter taste vomiting may occur. Cachets are expensive but tablets if properly prepared are quickly absorbed after oral administration; they are convenient and cheap for routine treatment, and for control of malaria on a large scale. Their therapeutic effects are not inferior. Subcutaneous injections find few advocates. Intramuscular injections should only be tried in those cases when the alimentary canal cannot tolerate quinine by the mouth. Under no circumstances should these latter be used for routine treatment of ordinary cases where quinine can be given by the oral route. Intravenous injections are preferable for severe and urgent cases, but as a routine treatment yield no better results than any other mode of administration. The physician treating the case will have to determine which is the best method to employ for the particular case.

As regards the time of administration of the alkaloid, we have already remarked that if quinine is given soon after a meal it hinders digestion by inhibiting the activity of the gastric and other ferments. It is advisable to give it 2½ to 3 hours after a meal when the gastric contents are acid, the digestion has been completed and the stomach is nearly empty. If given at this time it rapidly mixes with the contents of the stomach and passes into the small intestine, from which it is rapidly absorbed into the circulation. There is also less liability of its producing irritation of the stomach which it undoubtedly does when given on an entirely empty stomach early in the morning or a long time after a meal. James prefers to give quinine 2 to 3 hours before the paroxysm of fever is due. Intramuscular injections may be given at any time, but it is preferable to give intravenous injections when the stomach is not loaded.

(3) Other Cinchona Alkaloids in Malaria

Quinine till quite recently has been employed in the treatment of all forms of malaria, but Acton has shown that while it cures 90 per cent of malignant tertian infections if properly given, in the benign tertian infections the primary cures were not more than 30 per cent even after a two months' course. Cinchona febrifuge which contains all the alkaloids of cinchona bark gave a cure rate of about 50 per cent so that it can be reasonably concluded that there must be alkaloids other than quinine, which are responsible for this enhanced rate of cures. These other alkaloids were tested individually. Cinchonine and quinine in ten grain doses twice daily showed a cure rate of 60 per cent of benign tertian infections after a short course of three weeks.

Fletcher tested the action of different cinchona alkaloids individually in the treatment of malaria; his conclusions are as follows:—

Efficacy of individual alkaloids.

(1) In doses of 10 grains twice a day the 4 alkaloids, *i.e.*, quinine, quinidine, cinchonine and cinchonidine appear to be of equal value in bringing about the disappearance of malarial parasites in patients weighing 100 pounds.

(2) None of these alkaloids produce toxic symptoms when given in this quantity, not even cinchonine and quinidine.

(3) In doses of 5 grains twice daily, cinchonine does not appear to be quite so potent as quinine and quinidine.

(4) Cinchonidine sulphate is definitely inferior to other crystallisable alkaloids, when given in small doses.

(5) Quinidine sulphate acts better on the quartan parasites than quinine.

(6) Quinoidine in 5 grain doses does not cause the disappearance of the parasites; in 10 grain doses it cannot be tolerated as it produces severe nausea, vomiting and collapse.

It would appear from this that there is little to choose between the different crystalline alkaloids of cinchona bark so far as their action on benign and malignant tertian parasites are concerned. Fletcher's conclusions regarding the toxicity of quinidine are not borne out by our experience. It is liable to produce depression of the heart, and fainting and sudden deaths have been known to occur, especially in those suffering from emaciating diseases such as kala-azar.

It is evident that much waste has resulted in using only pure quinine, and cheaper and equally efficacious alkaloids might well be substituted in the treatment of ordinary cases of malaria while the more expensive and refined alkaloids may be reserved for severe types of cases.

Dale and James (1925) found the curative effects of quinine, quinidine and cinchonine is the same on all forms of malaria, and except for the depression caused by the last, there was no difference in toxicity. Ciuca (1925) made similar comparative tests with quinetum which is a form of cinchona febrifuge containing all the alkaloids but only 15 per cent of quinine and 5 per cent of quinidine. He found it to be as effective as pure quinine hydrochloride. In bird malaria the results with cinchonine, cinchonidine and quinidine ran closely parallel with those obtained in man. Sergents and Catnei (1925) found cinchonine and cinchonidine effective in removing malarial parasites from the blood and in reducing the size of the hypertrophied spleen; they found cinchonidine more powerful than cinchonine in splenomegaly. The Malaria Commission of the League of Nations (1927) stated that in doses of 1.0 gm daily, quinine, quinidine and quinetum are equally efficient in producing a clinical cure in malaria. Cinchonidine in doses of 1.5 gm equals the efficacy of other alkaloids.

Quinidine. The data available are not sufficient to justify a definite pronouncement whether quinidine is in all respects an efficient and quite satisfactory substitute for quinine against malaria. It has been considered by some to be as prompt in its action against benign and malignant tertian as quinine and as well borne, while others did not consider

it quite equal to quinine especially as regards preventing relapses; it was prone to cause more nausea. Fletcher (1925) considered quinidine as good as or even slightly better than quinine bisulphate in its effects. Sinton (1930) however thinks that quinidine has no more marked effect in producing a permanent cure in benign tertian malaria than has quinine. Quinidine is more expensive than quinine and has a more toxic action on the heart and this limits its use.

Cinchonidine. Cinchonidine sulphate is the least toxic of all the cinchona alkaloids. Investigations of Fletcher and others have shown that in 10 grain doses twice daily, it is almost as effective against malaria as quinine and on account of its lower toxicity it is well borne.

Cinchonine. Fletcher (1925) investigated its effect on behalf of the Medical Research Council and came to the following conclusions:—

(1) Cinchonine in doses of 0.1 grain per kilo body weight is less efficacious than quinine in reducing fever, and in clearing malaria parasites from the peripheral blood.

(2) Cinchonine in doses of 0.1 grain per pound weight of body is as effective as quinine.

(3) Cinchonine is not more toxic than quinine.

Quinoidine. This name has been given to the combined amorphous alkaloids which remain after all the crystallisable alkaloids have been removed from an extract of cinchona bark. Acton (1920) and Fletcher (1923) showed that it has no appreciable effect against malarial parasite in 5 grain doses twice daily.

In view of above facts it is hoped that the use of mixtures of the alkaloids will enable the treatment to be extended among the masses, as these mixtures are likely to be less expensive than pure quinine. The results of the trials of totaquina in the different countries have been analysed critically by Fletcher at Kuala Lumpur. Parasite-counting methods were employed in evaluating the treatment with totaquina and at the same time a control series of patients was treated with quinine. The records of the cases analysed, clearly show that totaquina acts like quinine as a potent remedy in all forms of malaria.

Hick and Diwan Chand (1935) performed experiments in India with two types of totaquina. The trial was carried out on the lines suggested by the Health Committee. More than 250 patients were treated and if the untreated control group is excluded, there was an average of 40 patients in each group and no group contained less than 31, so that in point of numbers it may be considered an important series. The quinine and both types of totaquina for these experiments were supplied by the Madras Government Cinchona Department. The composition of the two types are given below:—

	Type I per cent	Type II per cent
Quinine	32	19
Quinidine	1	4
Cinchonine	11	20
Cinchonidine	30	26
Amorphous alkaloid	15	19

The tablets of totaquina each contained 0.25 gm of the alkaloids. A dose consisted of one or more whole tablets together with one tablet cut down to a size estimated to complete the correct total dose. The conclusions of these workers are that under experimental conditions there was no distinct difference in efficacy between quinine and the two types of totaquina in clearing the blood of parasites either in benign or malignant tertian malaria.

Cinchona febrifuge, it has been pointed out, is a mixture of all the cinchona alkaloids of the *C. succirubra* bark. Residual alkaloids are a mixture of the alkaloids precipitated from the mother liquor after most of the quinine in the bark of *C. ledgeriana* has been extracted. To this latter certain proportions of quinine sulphate are added and the mixture is used as a substitute for quinine. Both these products are cheaper than quinine and under the name of cinchona febrifuge have been used in the treatment of malaria in India with gratifying results. The following mixture is prescribed:—

Cinchona febrifuge	10 grains.
Citric acid	30 ,,
Magnesium sulphate	60 ,,
Peppermint water	1 ounce.

One ounce of this mixture is prescribed three times a day 2½ hours after meals, for one week. It has been found quite effective, but it is liable to produce nausea and vomiting, as the amorphous alkaloids present stick to the mouth. The majority of patients, however, tolerate it well if it is taken at the right time, *i.e.*, 2½ hours after food.

Derivatives of quinine and cupreine in malaria. Henry and Brown (1923) showed that the toxicity of quinine base is greater to the paramœcium than that of its salts. Quinine base is also effective in clearing the blood of malarial parasites in human patients. Insoluble salts of quinine such as quinine tannate, though tasteless, are not very effective against malaria. Euquinine (quinine-ethyl carbonate) is tasteless and bulky, and it is effective in removing the parasites from the peripheral blood, as it is readily hydrolysed in the body to quinine itself. The hydroalkaloids such as hydroquinine hydrochloride, etc., are said to be more effective than quinine, by others less so. Cupreine sulphate in doses of 1 gm was found to be a good substitute for quinine in human malaria, but it is expensive and toxic. Good results have been with quinethyline and quinpropyline, in doses of 0.3 to 0.4 gm in human malaria and with these compounds and quinamyline in bird malaria. Ethyl-hydrocupreine or optochin has been tried in malaria with disappointing results.

(4) Quinine in other Conditions

Pneumonia

Quinine was used in the treatment of pneumonia over half a century, but more effective drugs are now available. Quinine finds no place now in practical therapeutics of pneumonia.

Common cold and influenza. Quinine is often used in the treatment of the common cold. It cannot reach the mucous membrane in strengths sufficient to produce antiseptic effects but it probably acts as a sedative.

Puerperal septicaemia and other conditions. Puerperal septicæmia was treated with 2 grains of quinine every few hours.

Exophthalmic goitre. Quinine and cinchona alkaloids are useful in this condition because of their vaso-constrictor properties on some vessels and secondly because of their effect on the heart. Besides this quinine retards all vital processes, it inhibits the conversion of glycogen into sugar and also limits protein metabolism. For this reason it has a beneficial effect in this disease where, owing to increased activity of the thyroid, the metabolism is markedly accelerated. A dose of 0.8 gm of quinine is given in two portions and increased gradually every day. The effect of quinine is said to be strengthened by combining it with 30 minims of extract of ergot.

Diseases of the circulatory system. Quinine has had the reputation for being a cardiac sedative for a long time. Oppolzer considers that along with rest and digitalis it is one of the three most powerful therapeutic agents in heart disease. It is said to have a damping effect on excessive digitalis action and is therefore often combined with it. It is claimed that a mixture of digitails and quinine produces better effects than digitalis alone. Quinine and quinidine have a powerful action in controlling cardiac arrhythmia and irregularities. These should however be used with great caution when there is true weakness owing to myocardial disease.

Other conditions. In lumbago, injections of quinine-urea-hydrochloride relieve pain. In the treatment of sciatica, injections of quinine-urea-hydrochloride into or about the sciatic nerve are recommended. One per cent solution is generally used and 15 to 30 ccm are injected at the point where the nerve lies over the neck of femur. The patient lies on the unaffected side with the affected thigh half-flexed. A point is selected somewhat lateral from the midpoint. The needle is pushed in for 3 to 4 inches and when it strikes the nerve a smarting pain is felt down the leg.

Quinine in 1 to 2 grain doses has been used with beneficial results in cases of vertigo combined with migraine. *Haemorrhoids* have been treated by injections of a 5 to 10 per cent solution of quinine-urea-hydrochloride. A perivenous sclerosis is said to be produced which progressively constricts the veins. The injections are painless.

In the treatment of *varicose veins* intravenous injections of quinine into the local veins are employed. Quinine is given along with ergot to cases of atony of bladder and cases of enlarged prostate who can not be operated upon.

Pruritis ani, vulvae and scroti have been treated by infiltration of the skin and sub-cutaneous tissue with 0.25 to 0.5 per cent quinine urea hydrochloride. Itching is at once

arrested and excoriations rapidly healed. One injection may suffice to keep down the condition for months. As much as 100 to 200 ccm of the solution may be necessary.

Venereal diseases. Quinine has been used as a prophylactic against contracting venereal diseases, because of its bactericidal action on the gonococci and spirocheticidal action. It is often combined with calomel and gelatine in a strength of 2.5 to .15 per cent.

Induction of labour with quinine. Quinine has been used for many years as a means of re-enforcing uterine contractions, especially when they are not strong. Most authorities agree that in therapeutic doses it does not excite the activity of the quiescent gravid uterus and therefore, it cannot be relied on for inducing premature labour. If however weak contractions are present they are intensified. The greater the tone and activity of the uterus the more rapid is the action of quinine. It is considered by some to be safer and its action more persistent than pituitrin. Various methods of giving quinine have been adopted.

Watson's method. Castor oil one ounce at 6 p.m., quinine hydrochloride 10 grains at 7 p.m., enema at 8 p.m., quinine hydrochloride 10 grains at 9 p.m., and again at midnight. If labour pains do not start by 7 a.m., on the next day, give ½ ccm of pituitrin intramuscularly and repeat every half an hour till labour commences. Balley (1926) recommends 2 ounces of castor oil to start with, and an hour after, ½ an ounce of quinine mixture containing 10 grains to the ounce. After one hour a simple enema is given. Two hours after the enema another dose of the mixture is given, this is repeated after 3 hours and then four hours later, making a total of 40 grains of quinine. The percentage of success with this treatment is greater than with pituitrin and the danger is less.

Quinine as a contraceptive. Quinine has been used as a contraceptive in clinical practice for a long time but during recent years it has been replaced by other drugs.

6. Toxic Effects

Lethal dose.

Quinine is one of the least toxic of the alkaloids, the lethal dose by the mouth in animals being 1.0 gm per kilo body weight. Frogs are killed by 1 mgm per gramme by the mouth and by about half this amount given by injection. Pigeons are not killed by 3 gm per kilo by mouth although 0.5 gm is fatal when given intramuscularly; rabbits are killed by 1.0 to 1.5 gm by mouth, by 0.25 to 0.5 gm subcutaneously and by 0.07 gm intravenously. A dose given into the artery of a limb does not kill but causes complete paralysis of the part supplied by the artery. It is suggested that this action is due to paralysis of the leucocytes which plug the vessels of the limb, causing palsy. The fatal dose in man is 8 to 15 gm by the mouth but in a few instances as much as 30 gm has been taken without producing death. Two to three grammes can be tolerated but 5 gm always produces toxic effects.

Toxic dose.

Toxic effects produced by cinchona alkaloids. The amount of quinine required to produce toxic effects varies enormously in different individuals. The solubility of the salt of the alkaloid employed, the vehicle in which the drug is given, the route of administration, the total quantity retained in the system, and the individual sensitiveness or idiosyncrasy of the patient to the drug, are some of the factors which play a part in all cases. In the majority of patients serious symptoms follow the administration of a total dose of 40 to 80 grains, while much smaller doses have produced toxic effects in sensitive individuals. Repetition of doses of quinine in patients who have suffered from cinchonism is not desirable.

The toxic effects produced by quinine can be discussed under the following headings:—1. Quinine idiosyncrasy. 2. Cinchonism.

(1) Quinine Indiosyncrasy

Sensititivity to quinine.

Some individuals show very peculiar susceptibilities in regard to quinine, even when such minute quantities as 1/16 grain are given. The symptoms which manifest themselves are a variety of skin eruptions such as scarlatina like erythema,

urticarial rashes, intolerable itching or bullous eruptions, which may be accompanied by fever. Other symptoms are dyspnœa, fever, nausea and vomiting. Sometimes there are giddiness and fainting. There may be extravasation of blood in the skin and perhaps in the intestines and kidneys; occasionally there is severe prostration which disappears by the following day, but reappears some weeks later when the dose is repeated. In some cases itching of the skin and vesicular eruptions are produced by contact with quinine solutions. Sometimes cinchonine or quinidine does not produce idiosyncrasy in cases in which quinine does so, and so either may be substituted. Idiosyncrasy may suddenly appear in persons who formerly have taken large doses of quinine without any disagreeable consequences. It may also declare itself by occurrence of toxic symptoms after small doses (deafness, amblyopia, etc.) which are usually produced after large doses. This idiosyncrasy is not commonly met with in the tropics, where large doses such as 20 to 45 grains are given in 24 hours and it appears to be similar to that of potassium iodide in which idiosyncrasy is more commonly seen with small doses than with large ones. If the serum of a person suffering from idiosyncrasy to quinine is injected intraperitoneally into a guinea-pig, the animal's susceptibility to quinine is greatly increased. Rarely, quinine produces a paradoxical or contrary action, producing an enormous rise of temperature accompanied by rigors instead of the expected fall. This phenomenon still remains unexplained.

Test and desensitisation.

In susceptible individuals local application of quinine solution (1 in 10 to 1 in 1,000) to the scarified skin produces a marked reaction which is not given by normal individuals. This may be used as a harmless test for quinine idiosyncrasy. The skin reactivity may be met with in the case of some lævorotatory alkaloids, *e.g.*, cinchonidine, ethylhydrocupreine, etc., and more rarely in dextrorotatory isomers such as quinidine, cinchonine, ethyl-hydrocupreidine. Sensitiveness to quinine is not congenital and does not exist in blood relations.

Desensitization. This has been proposed and tried successfully in some cases. The patient is given 5 mgm of quinine bisulphate plus 0.5 gm of sodium bicarbonate to start with, by the mouth. After 1½ hour 0.5 gm of sodium bicarbonate and 0.1 gm of quinine are given. The two doses of quinine are repeated daily, the desensitising dose being left at 5 mgm and the second dose being increased by 0.1 gm every day. Another plan is to give 0.5 to 1.0 ccm of adrenalin by injection, followed 20 minutes later by a small dose of quinine such as 0.05 gm. Next day the same injection of adrenalin is given, but the dose of quinine is increased.

(2) Cinchonism

Increased susceptibily.

This name is given to the early symptoms, chiefly connected with the central nervous system, which appear when quinine or other cinchona alkaloids are given in large doses. This should be differentiated from idiosyncrasy which is a condition of increased susceptibility and which is brought on by very small doses. Some individuals, however, are much more liable to get cinchonism than others. The power of producing cinchonism varies with the different alkaloids, cinchonine being the most powerful, next comes quinine, then quinidine and lastly cinchonidine. The association between the symptoms of intoxication and high concentrations of the alkaloid circulating in blood is very striking. When the concentration of quinine in blood reaches 10 mgm per 100 ccm symptoms of poisoning appear. The early symptoms are nausea vomiting mental

depression, headache (due to cerebral congestion), ringing in ears, giddiness and disturbed vision (due to selective changes in the vessels of the eye and ear). Minor symptoms may be somewhat diminished by bromide salts.

Toxic effects.

When a toxic dose of quinine is taken, first symptom is usually severe bursting headache, accompanied by loud roaring in the ears and deafness, which may be complete but is rarely permanent. There is loss of taste and smell, mental depression, photophobia, and later blindness, at first partial and then complete. With very large doses abdominal pains with nausea, diarrhœa, vomiting and general muscular weakness occur followed by difficulty of speech, confusion of ideas, somnolence, loss of consciousness, delirium, general paralysis, coma, at times convulsions, and finally death from collapse. Chills and cold sweats are also common, the pulse is rapid and weak and the respirations shallow. Large doses paralyse first the brain and respiratory centre and later the heart. The medullary centres as a rule are not affected till very late, and patients may recover even after very large doses. Quinine also produces skin rashes. There may be erythema, urticarial weals, swelling of the tongue and face, œdema of the eyelids, a scaly condition of the skin and a generalised itching eruption. It may also cause hæmorrhage from the nose, uterus and kidneys. Quinine amblyopia and amaurosis have already been referred to.

Quinine amblyopia.

Quinine interferes with the oxidation of the tissues and this oxygen lack causes a spasm of the retinal artery and leads to the changes associated with quinine poisoning. Two stages may be distinguished in the action of the drug; in the first stage the quinine, in its capacity as a general protoplasmic poison, acts directly on the retinal elements, and the second stage depends on the spasm of the retinal vessels and it is probably acute in onset.

Renal damage.

A fact of great clinical interest is that when large quantities of quinine are passed through the kidneys, temporary albuminuria is produced. Ramsden found that the proportions of quinine excreted diminished when the dose of the drug exceeded 2 gm by the mouth, and Nierenstein found that such a dose given orally or 1.2 gm given intramuscularly, frequently produced albuminuria. The kidneys can excrete very large quantities of quinine and as much as 2.2 gm have been recovered from the urine within a day. Quinine is usually excreted at a concentration of 600 mgm per litre in the urine which is about sixty times its concentration in the blood. Repeated administration of quinine does not increase the power of the body to destroy the drug, for the same proportion is excreted after prolonged administration. According to Marchaux 9.75 per cent of cases taking quinine have albumin in the urine. The dosage, mode of administration (intramuscular injections are more liable to produce it), diet, climate, etc., are all factors concerned. Sinton and Lal (1927) found that the incidence of albuminuria was much less when quinine treatment was combined with alkali than when quinine was given alone.

Apart from albuminuria produced by quinine, renal changes occur in malaria. From ancient times quartan fevers have been recognised to be the cause of nephritis. Giglioli (1932) has drawn attention to cases of sub-acute and chronic Bright's disease where symptoms develop with the appearance of the parasites in the blood and disappear on the administration of quinine.

(3) Visceral Degeneration

Degenerative changes.

Cornwall (1920) showed degenerative changes in the suprarenals and the kidneys, and increased degeneration of the erythrocytes in the spleen after large doses of quinine in rabbits. That quinine has toxic effects on the liver has been experimentally proved. Intravenous injections of moderate doses in rabbits produce progressive degeneration of liver cells, which increases with the increase of dosage. Quinine has a certain amount of hæmolytic effect on the blood cells especially in high concentration. It modifies the response to other hæmolytic agents such as acid solutions.

Macht and Teagarden (1923) showed that quinine and quinidine are more toxic to rats exposed to sunlight or to a quartz lamp than to those kept in the dark. Exposure of quinine solution to ultra-violet light produces chemical changes which render it more toxic to paramœcia *in vitro* and also more effective in bird malaria (1920).

The toxic effects of the other cinchona alkaloids are similar to those produced by quinine, but they show important differences that might make them therapeutically less desirable. Cinchonine and cinchonidine in large doses produce tonic and clonic convulsions, the site of their action on the brain being located below the optic thalami. Quinidine has a stronger depressant and paralysing effect on the heart. Cupreine is about half as toxic as quinine. Cinchonine is more toxic than quinine and produces salivation, hallucination and epileptiform convulsions. It has little effect on the blood pressure.

The grave effects of cinchonism are only met with when the dose of quinine is increased beyond 40 to 60 grains a day. Such large doses were prescribed at one time by practitioners in India and were often continued for long periods. These undoubtedly do harm. As a rule a maximum dose of 20-30 grains per day, according to the weight of the patient, divided into 3 or 4 doses, are quite sufficient in an adult to control the fever. Such doses are quite safe and effective and as a rule cause no untoward symptoms. There is no particular point in giving very big doses by the mouth as the intravenous and intramuscular method can always be resorted to when there is doubt regarding its absorption from the gut.

A form of toxæmia occurs in patients taking large doses of quinine for prolonged periods. In the old days patients who had suffered from malarial fever were put on large doses of quinine for periods ranging from 1 to 3 months, and this condition was not infrequently met with. The patients looked pale and anæmic, they were listless, had no inclination to make any exertion and had lost their appetite for food. There was generally a jaundiced tinge about the conjunctiva and the temperature was usually subnormal. All these symptoms were put down as after-effects of malaria, but they were due to the action of quinine on the gastro-intestinal tract and other organs, because stoppage of the drug or decrease in the dose rapidly improved the condition. The toxic effects of quinine have been attributed by some to the formation of a body called quinotoxin which is formed by the action of free organic acids on quinine, and it is suggested that such conditions arise in the gut. It is however found that this substance has a very low toxicity and could not be responsible for these effects.

(4) Treatment of Cinchonism

Numerous drugs have been tried to obviate cinchonism, and of these caffeine given subcutaneously or intravenously shortly before the administration of quinine is the best. To relieve headache phenacetin has been tried but it may depress the heart. If nausea is present 20 to 30 grains of sodium bicarbonate in a tumblerful of hot water is useful. Bromide of quinine is said to produce fever, symptoms of cinchonism, and therefore quinine has often been prescribed with hydrobromic acid. Some suggest 7½ grains of peptone (Witte) orally, half an hour before quinine is taken, because of its anti-anaphylactic effect; others recommend use of adrenaline. A skin reaction by scarification with 10 per cent solution of quinine sulphate has been described. In all cases with toxic manifestations, administration of quinine should be stopped and atebrin or paludrine substituted. This enables patients sensitive to quinine to be made fit for service in the tropics.

7. Mode of Action of Quinine

Direct effect.

Rationale of action of Cinchona alkaliods in malaria. The cinchona alkaloids have a remarkable action against malaria. They kill the parasites responsible for the disease without injury to the host. If blood containing parasites is examined on the warm stage of a microscope, in the presence of minute quantities of quinine, movements of the parasites are arrested; if however the drug is given by the mouth, three hours elapse before the endocorpuscular forms become immobile, granular and lose their affinities for certain stains. It has been found that quinine does not attack the parasites with equal virulence in all the stages of development. The asexual forms as a rule are much more vulnerable; the sexual forms of *P. falciparum* appears to be hardly touched. The action of cinchona alkaloids controlling attacks of malarial fever is not understood. There are many points in connection with directing the attack of these alkaloids upon the most vulnerable stage of the parasite, about which we have not sufficient knowledge.

The concentrations of quinine in the blood attained after therapeutic doses are not sufficient to kill the malarial parasites by their direct action. Other factors therefore must come into play.

Concentration in blood.

Ramsden, Lipkin and Whitley (1918) found that quinine was present in the blood in a strength of 1 in 100,000, seven hours after administration. In a number of human cases treated with intravenous injections no direct correlation was found to exist between the concentration of quinine in the blood and the disappearance of the parasites. From the blood, a certain amount, varying with the individual, but averaging about 40 per cent of

the total amount administered, is eliminated by the kidneys within three to twenty-four hours. Very little passes out in the fæces unless there is diarrhœa. The balance is fixed in the tissues, more in some than in others. It is apparent from these studies that quinine makes only a brief stay in the blood circulation, and is only present here in minute traces, the blood corpuscles taking up very little. Acton and King (1921) concluded that the distribution of quinine between plasma and corpuscles was about equal. The question is of some importance the malarial parasites being intracorpuscular.

The following concentrations of quinine were found in the blood by Acton and King after 15 grains were given on an empty stomach.

Quinine

Interval after administration (hours.)	Concentration of quinine in blood	Amount in blood (mgm.)	Per cent. of the dose taken.
1	1 in 150,000	33	3.3
2	1 in 187,000	27	2.7
3	1 in 225,000	22	2.2

Hydroquinine

1	1 in 250,000	20	2.0
2	1 in 280,000	18	1.8

After intravenous injection, a concentration as great as 1: 20,000 may be obtained but rapid elimination from the blood stream takes place. It is extremely difficult to bring the concentration in the blood up to 10 mgm per litre, the highest figure, so far obtained experimentally being 16.6 mgm which was associated with very severe symptoms of poisoning. Even this concentration maintained for 48 hours failed to effect a radical cure of benign tertian infection.

Malarial parasites are hæmosporidia and as far as is known live only in the red blood cells; they are not found in the plasma in a free condition or in the cells of the tissues. The only occasion on which they are free is when the mature schizonts rupture and they attach themselves to fresh erythrocytes. It is possible that the cinchona alkaloids may destroy them in one of the following ways:—

Possible explanation.

(1) By making the erythrocytes distasteful for habitation of the parasite in the same way as *E. histolytica* will not ingest emetinised blood cells. The merozoites are in this way prevented from penetrating the red blood cells and perish because they are deprived of the only food they can live on.

(2) By parasiticidal action when the parasite is in the red cells. It has been said that the action is most marked on the youngest forms, less on the mature forms and very little on the gametocytes. The intra-corpuscular forms are not more vulnerable to quinine than the extra-corpuscular forms. Craig after careful study of the action of quinine on malarial parasites in fresh and stained specimens came to the conclusion that it affects the parasite injuriously in all stages in man except just prior to sporulation. Some of the recent work, however, throws doubt on the parasites being in the red corpuscles; there are some indications that they may lie on the surface.

(3) By the destruction of the merozoites when they are passing from one corpuscle to another. The sporulating body is not affected by quinine and sporulation occurs, but most of the spores are said to be destroyed by the drug in the blood plasma (Craig). The disappearance of the young forms indicates that they are the forms most vulnerable to the action of these alkaloids. Once the parasite gains entrance into the erythrocyte, probably schizogony can be completed in spite of quinine. This, however, is not the whole explanation, for if it were true, quinine would be much more efficacious, during the time of sporulation.

(4) Morgenroth (1918) believed that malarial parasites are unable to enter red blood corpuscles which have been treated with quinine. It has been recently shown that quinine tends to condense on the surface of solutions, or at the interface, to such a degree that it forms a rigid film. If a tough film were formed on the blood corpuscles, this might protect them mechanically against the entrance of the parasites.

(5) By the formation of potent decomposition products in the body. It has been shown in the case of the organic arsenicals that the concentration required to kill the parasites

outside the body appears much higher than the concentration which can be conceived to exist in the blood stream. This is attributed to their transformation into more effective compounds in the body. In the case of alkaloids like quinine, emetine, etc., the assumption of toxic transformation is so unsupported by the chemistry of these agents that it appears to be improbable. A substance named quitenine is formed from quinine by the action of the liver. Quitenine however has no action on the malarial parasites either in birds or in man. Quinine does not form any antimalarial compounds with the tissues on incubation. It appears probable that the parasiticidal effect is not a direct one.

(6) Another characteristic of the cinchona alkaloids has been shown to be their property of interfering with certain enzyme reactions. This property of enzyme inhibition shows considerable selectivity which would be more consonant with the specificity of antimalarial action. This would then be conceived as interference with a metabolic process that is essential to the developmental cycle of the plasmodia.

(7) It is quite possible that quinine acts mainly on the young forms in the course of their passage from one erythrocyte to another or when they are adherent to them or before they have actually penetrated into the red cells, *i.e.*, whilst the parasite is in contact with the alkaloids circulating in the plasma. Quinine and the other alkaloids of cinchona, even in high dilutions, inhibit the movements of malarial parasites and may even paralyse them. It is even possible that quinine may condense on the parasites and thus hinder their movements. This action may prevent the parasites from penetrating further into the red cells and thus deprive them of the only food they can live on.

Although the exact method of action of quinine on malarial plasmodia is still unknown, Lourie (1934) has brought forward evidence to show that quinine acts by inhibiting reproduction, retarding the asexual cycle and decreasing the number of merozoites. The mechanism is thus quite distinct from that of acquired immunity where the parasites are killed without any evidence that the rate of reproduction is inhibited.

There is no evidence that the prolonged administration of quinine or plasmochin leads to drug resistance on the part of the plasmodia.

Gametes in blood.

The action of cinchona alkaloids on the gametocytes. Crescents appear suddenly in the peripheral blood on the 11th day of disease, increase to a maximum and then decrease. The average duration of a crescent wave is 14 days, but crescents may persist for 66 to 128 days, when there are frequent relapses. Crescents are most plentiful in new cases and in relapses and are fewer in number in chronic, latent and recrudescent malaria. In acute cases of malaria administration of quinine for short periods is said by some to favour the appearance of gametocytes in the peripheral blood. Two hypotheses have been advanced to account for this:—(1) That the administration of quinine expels the gametocytes from the deep viscera to the peripheral blood and (2) that it creates an environment which favours the development of increased numbers of gametocytes. According to others, quinine in the early stages does not increase but tends to inhibit crescent production.

Every division of parasites produces new gametocytes, the number of the latter increases progressively in the body as a result of repeated attacks. The sooner malaria is treated with quinine and the bouts of fever are reduced, the less the number of cycles that a parasite will undergo, thus indirectly reducing the number of gametocytes.

The formation of resistant forms may be nature's method of self-preservation of the species and occurs in all forms of free living unicellular organisms. When the environment is favourable so far as nutrition is concerned, multiplication takes place asexually; when the environment becomes unfavourable, the organism after conjugation assumes the cystic or resting stage until conditions are once more favourable for their asexual multiplication. On the other hand, many malariologists are now abandoning the view that crescent production is a response to unfavourable environment. Crescent production is at its maximum in infants and very young children who are extremely susceptible to malaria. Occasionally also, it may occur at the very beginning of an attack of subtertian malaria. When the gametocytes are present in the peripheral blood, the mosquitoes become infected.

Effect of quinine.

The cinchona alkaloids have been shown to have little or no action on the gametocytes of malignant tertian parasites in the blood and only slight action on

those of benign tertian. Administration of quinine however in doses of 20 to 30 grains a day reduces the number of crescents, probably by cutting off the source of supply by killing the asexual forms. According to James (1924) the development of gametocytes in all three species is not morphologically affected by quinine. The life of a red cell is said to be 30 days, though estimates vary considerably; it follows therefore that crescents cannot live longer unless the dead red cells adhere to the crescents. Muhlens and Kirchbaum (1923) transmitted subtertian malaria through *A. maculipennis* to a fresh case, the donor at the time of infecting the mosquito being under the influence of quinine. It has also been shown that gametocytes developed under administration of quinine are viable. The gametocytes of malignant tertian type after administration of quinine will still be capable of infecting mosquitoes and spreading infection, although the patient himself may be freed from fever. On the other hand although quinine will not cure a benign tertian infection it will emasculate the benign gametocytes so that there is no risk of their passing on to others (Meyer and Wenyon). There is no other drug except plasmochin which has a direct action on the crescents and destroys them. Quinine in very low concentration stimulates metabolism and increases the rate of multiplication of parasites (Acton and Chopra 1927), but in the dilutions in which it circulates in the blood after therapeutic doses it decreases the rate of multiplication and therefore few merozoites will be formed.

Bass (1921) showed that quinine bihydrochloride in concentrations of 1 in 4,000 when incubated for 5 hours with the schizonts of *P. falciparum* may produce degeneration of the parasites. Direct action of quinine upon the plasmodia is however negativated by the observations made by Muhlens and Kirchbaum (1923) and later confirmed by other workers. It was shown that defibrinated tertian blood mixed with quinine hydrochloride in a strength of 1 in 5,000 still produced infection after 12 hours, exposure in patients suffering from general paralysis of the insane. The blood left for five hours in contact with 1 in 2,500 quinine hydrochloride will still infect these patients. Quinine given to the donor does not render the blood materially harmless to the recipient, and mosquitoes can be infected from the blood of patients taking quinine which shows that the action of quinine is indirect. Once the parasites enter the red blood corpuscles, they are not much affected by the action of quinine, and this is a point in favour of the paralytic theory of the merozoites. Clinicians know that when quinine is administered after sporulation is completed, it does not prevent the next attack.

Mechanism of cure.

The mechanism of cure of malaria cannot, therefore, be by quinine acting directly on the parasites. Lipkin (1919) suggested that possibly a metabolite of quinine was the active agent. It has also been suggested that possibly an antigen from destroyed parasites stimulates immune-body production and helps to overcome the infection. Taliaferro, Krishnan and others have shown that the cells of the reticulo-endothelial system engulf and destroy the parasites directly and upon their number and functional efficiency depends the immunity in malaria.

Summary of treatment of malaria with cinchona alkaloids

Summary.

The cinchona alkaloids have no action on the sporozoites injected by the mosquitoe, nor have they any effect on the extra-erythrocytic forms (if they exist in man). They act on the trophozoites, schizonts and merozoites. Although they may not have any direct destructive action on the gametocytes, they act indirectly by destroying the schizonts from which the gametocytes develop.

The cinchona alkaloids are best suited for treatment of malaria when it cannot be carried out under proper medical supervison. They are indicated for mass treatment and for treatment in children (under six years) or where there is history of mental or nervous disease. Patients coming from an endemic area should be given a full course of cinchona alkaloids (10 days at least) after return.

Although all the crystalline alkaloids of cinchona bark are effective, quinine is preferred for an acute attack in an adult, quinine sulphate or hydrochloride is given by the mouth in solution form or as tablets, in doses of ten grains twice daily after a preliminary purgative. Larger doses are unnecessary and in small persons even fifteen grains are effective. The course should last for 7 to 10 days and the alkaloid should be combined with alkali treatment. If relapses occurs the course should be repeated each time. Instead of quinine tota-quinine may be used.

In pernicious malaria, quinine in doses of 7 to 10 grains is given immediately by the intravenous route. One to three such injections will suffice to control symptoms, and after that quinine is given by the intramuscular or oral route.

For chemoprophylaxis, six grains daily are advised throughout the stay in a hyper-endemic area and for at least ten days after return if not longer.

Quinine should be preferably used by the oral route. The parenteral routes should only be used if oral administration is not possible.

8. Preparations of Cinchona Alkaloids and its Derivatives

Cinchona Bark, dose 5 to 15 grains. *Extract cinchona* (contains 10 per cent total alkaloids) dose 2 to 8 grains. *Extract cinchona liquid* (contains ¾ gr. of the alkaloids in 15 minims). Dose 5 to 15 minims. *Compound tincture of cinchona* (contains 0.5 per cent. of total alkaloids), dose ½ to 1 drachm (2 to 4 ccm.). *Cinchona febrifuge* is a mixture of the total alkaloids of cinchona bark of varying composition. It has been extensively used in India for the last 50 years with satisfactory results. It is cheap and suitable for mass treatment. It has recentlly been standardised by the Malaria Commission of the League of Nations.

Totaquina. A mixture of the alkaloids from the bark of *Cinchona succirubra* and other species of cinchona. It contains not less than 70 per cent. of crystallisable cinchona alkaloids, of which not less than 1/5th is quinine. Dose 1 to 10 grains.

CINCHONA ALKALOIDS AND THEIR PREPARATIONS

A. Crystalline alkaloids.

Quinine hydrobromide occurs in white acicular crystals, soluble 1 in 40 of water and contains 76.6 per cent of quinine. It is said to lessen cinchonism and is valuable in acute rheumatism. Dose 5 to 15 gr. *Quinine bihydrobromide* contains 60 per cent of quinine, soluble 1 in 7 of water and is suitable for subcutaneous and intravenous injections. It is non-irritating and the additional hydrobromic radicle tends to prevent cinchonism. Dose 5 to 10 grains.

Quinine hydrochloride contains 81.7 per cent of base. Solubility 1 in 32 of water and in 1 in 2 of 90 per cent alcohol; used for subcutaneous injections. Dose 1 to 10 gr. It is mixed with theobroma oil and forms a part of many contraceptive preparations. Quinine hydrochloride is contained in *Tincture quinine,* the dose of which is ½ to 1 drachm and *Vinum quininae,* dose ½ to 1 ounce.

Quinine bihydrochloride. contains 81.6 per cent of quinine; solubility 1 in 0.6 of water. It is considered a good salt for intramuscular and intravenous injections; the hydrochloride radicle is also not as irritating as the sulphuric radicle to the stomach. Dose 1 to 10 gr. For intravenous injections some use 1 to 300 solution, others 10 per cent solution. Half to 1 per cent produces local anæsthesia when injected; for painting on mucous membrane 10 per cent solution is the best. Sterules of various sizes are on the market.

Quinine hydrochloro-carbamide or urea-quinine occurs in small prisms; solubility 1 in 1 of water; contains 59.2 per cent of quinine. Dose 5 to 15 gr. Used to produce local anæsthesia in 1 to 3 per cent solutions; anæsthesia persists from 4 to 6 hours to several days.

Quinine phosphate contains 72.8 per cent of quinine base. Dose 1 to 6 gr.

Quinine salicylate occurs in white crystals; sparingly soluble in water; contains 68.8 per cent of quinine base. Useful in sore throat.

Quinine acetyl salicylate contains 64.3 per cent of quinine. It is a useful antipyretic and antiseptic compound. Dose 1 to 5 gr. It is prescribed in painful sore throat.

Quinine sulphate contains 73.5 per cent of quinine base. Dose 1 to 15 gr. It occurs in white crystals, solubility 1 in 800 of water. It occurs in a number of preparations. *Ammoniated tincture of quinine* is a solution in 60 per cent alcohol to which ammonia is added; the precipitate formed is suspended in tragacanth. *Quinine bisulphate.* Dose 1 to 10 gr.

Quinine tannate contains 30 to 35 per cent of quinine base. It is tasteless and is recommended for children. Absorption is uncertain. It splits up slowly in the intestine and is therefore not prompt in its action. Dose 1½ to 15 gr.

Quinine ethylcarbonate or euquinine occurs in white needles having a melting point not below 95°C. It is sparingly soluble in water but is more so by addition of dilute acid, easily soluble in alcohol. It is practically tasteless. Eight grains of euquinine are equivalent to 5 grains of quinine sulphate. It is rapidly absorbed and appears in the urine soon after injection. Dose 3 to 16 grains.

Aristo-quinine or *aristochin* is insoluble in water and is tasteless. It contains 96.1 per cent of quinine. Dose 1 to 10 gr. It has been used against malaria, influenza, and in small doses in pertussis.

Soloquinine is the name given to quinine salicylic ester.

Chenophenin is phenetidin-quinine-carbonic ester. Both these compounds exhibit the action of both the constituents of the drug. *Collobiase of Quinine* is a preparation in which quinine is kept in a colloidal state in combination with arabinic acid. It contains 30 per cent of quinine base. It can be given intravenously and subcutaneously.

Collosol quinine or the alkaloid in colloidal condition has been introducced.

Tests for quinine.

1. *Fluorescence.* The alkaloid is dissolved in dilute sulphuric, acetic or tartaric acid (the test tubes should be made of transparent glass or silica). One mgm of quinine can easily be detected in 4 ccm of solution. Fluorescence is visible in acid solution containing 1 in 200,000 of quinine. The fluorescence may be masked by the presence of chlorides and hence the test is not a very useful one.

2. *'Thalleioquin' reaction.* To 10 ccm of the solution of quinine add 3 ccm of chlorine water or 0.5 ccm of saturated bromine water. Shake well, and then add one drop of strong ammonia solution or sufficient to render the liquid distinctly alkaline. If the proportion of quinine exceeds about 1 per 1000, a green substance is precipitated. In more dilute solutions, a deep green colouration is produced. One in 5,000 quinine solution is detected by this test and if bromine is used instead of chlorine, quinine can be detected in a still higher dilution (1 in 20,000). This test is given by quinine, quinidine, and the hydroquinine alkaloids, and the decomposition products quinotoxin and quitenine. The cupreine series also give this test. It is not given by the members of the cinchonine series or their decomposition products. In 10 ccm of solution 0.25 mgm of quinine can be detected with ease.

3. *Tanret-Mayer Test.* The reagent is prepared by dissolving 1.45 gm of $HgCl_2$ in 80 ccm of distilled water and 5 gm of KI in 20 ccm of distilled water and mixing them in a 100 ccm measuring flask. The $HgCl_2$ solution is poured into the KI solution agitating all the time; water is added to make it up to 100 ccm. This reagent precipitates all alkaloids and gives a good rough test for the presence of quinine in urine. The urine is acidified and boiled to free it from albumin and is filtered. Five ccm of this is taken in a test tube and Tanret Mayer Reagent is added. A precipitate shows the presence of the alkaloid If there is no distinct turbidity the patient has not taken quinine. Mann suggests taking 50 ccm of urine and adding 2.5 ccm of lead acetate solution (lead acetate 25 gm, glacial acetic acid 2.5 ccm, water to 100 ccm); mix thoroughly and add 2.5 ccm of saturated aqueous solution of ammonium sulphate; filter till a clear lead-free filtrate is obtained. Receive a drop of filtrate on a paper moistened with a sulphide to test for lead and also boil to test for albumin. To 10 ccm of this add 0.5 ccm of Mayer's reagent. An opalescence or turbidity indicates the presence of quinine. Very slight traces of quinine may only show after the mixture has stood for 10 to 15 minutes.

Another simple test for quinine in urine is to take 10 ccm of leadfree urine as obtained in the above test and adding to it 6 drops of 1.0 per cent solution of picric acid. A cloud forms if quinine is present, disappearing on heating and reappearing on cooling. A precipitate

due to albumin increases on heating and falls on standing. The test responds to 3 grains of quinine taken an hour before examination.

Quinine mixtures used in dispensaries in this country are not infrequently below strength. Two methods for testing the approximate quantity have been described.

(1) *Megaw, Ghosh and Chatterjee method*:

Requirements:—(1) A supply of long narrow test tubes of equal calibre (5 to 7 mm diameter if possible). Wider tubes may be used but they would necessitate the use of larger amounts of the solutions. Calibrated and graduated tubes such as centrifuge tubes are preferable if available.

(2) Reagent:—Twenty gm of Merck's pure phosphotungstic acid is dissolved in 100 ccm of 12.5 per cent sulphuric acid. The dilute H_2SO_4 is made by mixing 5 ccm of B.P. concentrated sulphuric acid of 1.84 specific gravity with 50 ccm. water, cooling, and making up to 70 ccm with more water.

(3) The stock solution which is to be tested. (This should be diluted if it contains more than 20 grains of the alkaloid in each ounce; in this case, the control solution should be diluted in the same proportion).

(4) A freshly prepared solution should be made up in one's presence, using the same amount of quinine or cinchona febrifuge as is supposed to be present in the stock solution, the powders which are in stock in the dispensaries being employed. In cases of doubt as to the purity of the powdered alkaloid in stock, another solution should be made using powders of known purity. This will serve as a control of the quality of the stock powders.

Method:—In one tube take 1 ccm (or one part) of the stock solution, in another tube take exactly the same amount of the freshly prepared solution (a third tube may be used for the solution made from alkaloids of known purity). Measurement by a pipette is desirable but with reasonable care equal quantities can be measured.

To each of the tubes add 2 ccm (or 2 parts) of the reagent and 1 ccm (or 1 part) of water. Mix thoroughly by rolling the tubes between the hands for about a minute keeping them in a vertical position. Do not stir or shake the solutions. Allow the precipitates to settle for 2 or 3 hours and compare their heights. Any great variations will indicate the necessity for an accurate quantitative examination of the stock mixture.

If centrifuge tubes and a centrifuge are available the examination can be completed in a few minutes, but the test has been specially worked out so that it can easily be carried out with the simplest possible equipment.

The test solution ready made can be obtained from any reliable chemist by supplying him with the formula. It keeps indefinitely. It is essential that the tubes employed should be of equal calibre and the quantity of the quinine solution in each tube must be the same. The total volume should be such as to give a 5 per cent solution of sulphuric acid, at which the reagent acts best. No claim of great accuracy is made for this test, but it will be found useful as a rough check also used to detect any serious deficiencies in the purity of the powders from which the stock mixtures are made. Messrs. Boots Pure Drug Co., Ltd., have prepared a handy 'Quin-Iodide Test Outfit' based on this method.

Quinidine. This isomeride of quinine is contained in small quantities in most cinchona barks, but especially in *C. pitayensis, C. amygdalifolia* and *C. calisaya.*

Properties. Quinidine is alkaline in solution and behaves as diacidic base forming two series of salts. The neutral and acid sulphates are soluble in water. The acid hydrochloride is sparingly soluble. The melting point of the base is 171.5°C and the optical rotation is +236.7° in 97 per cent alcohol.

Tests. It is fluorescent in dilute sulphuric acid and gives the thalleioquin test. It differs from quinine in being dextrorotatory, possessing a sparingly soluble hydriodide (1 in 1250 at 12°C) and a neutral sulphate fairly soluble. The tartrate is soluble and this fact is utilised in the separation of cinchonidine.

Dose. It has a much more powerful action on the heart than quinine, 5 to 7½ grains is the usual dose given two or three times a day orally.

Hydroquinidine is the hydrocompound of quinidine and occurs in commercial quinidine.

Cupreae cortex or *Cuprea bark* is obtained from *Remijia pedunculata* and other species. It contains quinine and an allied alkaloid, cupreine. A number of derivatives of the aliphatic series have also been prepared. It will therefore be useful to mention some of these preparations in this connection.

Methyl hydrocupreine hydrochloride is also known as hydroquinine hydrochloride. Dose 4 to 12 gr (0.25 to 0.8 gm) for adults in malaria. In whooping cough in children it is given in 1 to 5 or 6 gr intramuscularly, according to age.

Ethyl hydrocupreine or *optochin,* is a white amorphous powder with a bitter taste, almost insoluble in water, soluble in alcohol, ether, chloroform and dilute acids. Dose 3 to 4 gr (0.2 to 0.25 gm). *Ethyl hydrocupreine hydrochloride* is given in the same dose as the base and it is soluble in water.

Iso-amyl hydrocupreine. This derivative according to Dixon (1920) is 10 to 20 times more powerful than quinine in the destruction of protozoa, 0.1 per cent solution acts as a germicide and as a local anæsthetic.

Iso-octyl hydrocupreine or *vuzin* is said to have a specific action on *C. diphtheria* and enough can be given in medicinal doses to clear this organism from the blood.

9. Plasmoquine (Plasmochin, Pamaquin)

Plasmoquine is a quinoline derivative known as pamaquin or præquin, British; rhodoquine French is an aminoquinoline derivative of methylene blue.

Introductory.

Owing to the high price of quinine, its bitter taste, and its failure to remove crescents from the blood, attempts were made to find stronger and better drugs. These researches led to the synthesis of various antipyretic drugs such as antipyrin (1884), phenacetin (1886), etc. In 1926, Professor Schulemann and his colleagues chose methylene blue, which had been shown to have anti-malarial, properties, as the starting point for investigation and they prepared a large number of compounds. One of these 6-methoxy-8-N-4-diethylaminoiso-pentylaminoquinoline tested by Roehl was found to be particularly effective against bird malaria. Plasmoquine although orginally derived from methylene blue, has a constitutional formula, which has analogies to that of quinine. Its chemotherapeutic index is 1:33 as compared with 1:4 for quinine. The first announcement concerning it was made in September, 1926. It has also been called 'plasmochin' and later in English-speaking countries it was called plasmoquin or plasmoquine. 'Beprochin' is a substance of a similar nature. Fourneau 664 is another compound allied to plasmoquine. It had been shown that apart from arsenicals, the substances effective against malaria in man are effective against bird malaria also. This process was reversed in the case of this drug and it was found that a drug useful in bird malaria was found to be beneficial in malaria in man. Roehl found that plasmoquine had a definite destructive action on the plasmodium in the birds and was nearly 60 times stronger and more effective than quinine. One ccm of 1 in 50,000 solution of plasmoquine given daily for 6 days, delays development of plasmodium in canaries, while the same quantity of 1 in 800 solution will be necessary in man. Later, it was found that *Haemoproteus* may be temporarily removed from the peripheral blood of thrushes; in pigeons plasmoquine removes the gametes of *Hæmoproteus* from the peripheral blood stream, though they return again after a few days. Plasmoquine destroys the gametocytes, but not the schizont.

(1) Pharmacological Action

On non-pathogenic protozoa such as paramœcia, plasmoquin like quinine has a lethal action but in double the concentration of the latter drug. In the dark however, plasmoquin is more effective than quinine as light seems to activate the latter drug. When the two drugs are mixed they appear to reinforce each other's action and the toxic action on paramoecia is intensified.

Circulation.

Eichholtz (1927) studied the action of this drug on the circulation. In cats, doses of 2.5 to 5 mgm per kilo. of body weight caused formation of methaemoglobin. In cats, dogs and rabbits, intravenous injections produced cardiac incoordination. In large doses (1 to 3 mgm per kilo of the body weight) the drug increases the refractory period of the ventricles and heart-block may be produced. The heart becomes irregular, there may be duplication or suppression of the systole. With higher doses the heart is depressed and the blood pressure falls. Adrenalin brought about normal rhythm in the heart poisoned with plasmoquin and produced the usual rise of blood pressure. Like quinine it does not decrease the number of white blood corpuscles but in a few cases actual increase was noticed in man. Quinine in certain doses is said to prevent the toxic effect produced on the heart and that is the reason why plasmoquin and quinine are combined in the form of plasmoquin compound tablets. This however seems to be doubtful. Leucocytosis sometimes follows the administration of plasmoquin, many young undifferentiated leucocytes being present. It is therefore preferred in malaria complicated with pneumonia.

Uterus.

Chopra and his co-workers (1933) have shown that plasmoquin in therapeutic doses has no action on the uterus. In large doses it produces contractions of the isolated uteri of cats and guinea pigs. That plasmoquin prevents the stimulant effect of quinine on the uterine contractions is not borne out by experiments. When proportionately equal doses are employed the action of plasmoquin in the cat and man is similar.

Plasmoquin is toxic to the central nervous system.

Excretion.

The fate of plasmoquin in the body is not known. That it is partially excreted in the urine there is little doubt. Its presence can be actually detected in the urine.

Test for plasmochin in body fluids.

Nandi & Dikshit (1938) have evolved a test for detecting the presence of plasmoquin in blood and tissues. Plasmoquin can be detected in dilution of 1 in 200,000 by a colorimeter and up to 1 in 1,000,000 by a spectrophotometer. The test cannot be applied in case of urine where some interfering substance seems to be present. It is quite simple; a few drops of Folins phenol reagent are added to the aqueous solution of plasmoquin and then sodium bicarbonate is added to make the mixture strongly alkaline. A blue colour develops slowly becoming maximum in the course of thirty minutes.

Toxicity.

The lethal dose in rabbits is 3 to 5 mgm per kilo body weight intravenously, 20 mgm subcutaneously and 225 mgm by the mouth. In the cat it is more toxic; 5 mgm per kilo intravenously is fatal while the same amount will also kill when given subcutaneously and 7.5 mgm is fatal by the mouth. Cats recover more quickly than rabbits after sublethal doses. Death occurs with symptoms of dyspnoea, asphyxia, bradycardia and arhythmia, with methaemoglobinaemia. It has been shown that plasmoquin forms methaemoglobin with the blood of the sheep, ox, horse, cat, rabbit, dog and man in vitro. In man one-third of the oxygen capacity of the blood may be lost as the result of this conversion. In acute poisoning the symtoms appear in the following order. First there is paralysis of the vasomotor system with fall of blood pressure, slowing and then complete cessation of respiration; the cardiac rhythm remains undisturbed till the end. Lethal and therapeutic doses in man are not widely separated.

(2) Effectiveness Against Malaria

The drug was first tried on man by Sioli (1926) who cured several patients artificially infected with malaria in the course of treatment for general paralysis. Muhlens and Fisher (1927) tried the drug in patients suffering from malaria. In a series of 172 cases they found that 0.08 to 0.1 gm per day was an effective dose in terian and quartan malaria. Such doses, however produce toxic effects. The drug was later tried in most of the malarious countries in Europe, the main results of these observations being that plasmoquin in doses of 0.06 gm per day given daily for one week, and for 3 days in the week for the next five weeks was effective in curing malaria. This was the method recommended by the manufacturers. Sinton (1928) gave the drug daily for 28 consecutive days and found this method more effective though more toxic. The conclusions arrived at were that the destructive action of plasmoquin is restricted to infections by *P. vivax* (benign tertian) in all phases, and that while it has no action on the schizonts of *P. falciparum* (malignant tertian) it has a definite action on the crescentic gametocytes of this organism. Plasmoquine is perhaps the only one of the older drugs which has some action on the extra-erythrocytic phase.

B.T. and quartan infections.

Both benign tertian and quartan parasites disappear from the blood more rapidly than the sub-tertian, and cure is said to be more readily effected in these infections than with quinine. After doses of 0.01 gm, 4 or 5 times a day or 0.06 gm daily, the fever usually disappears from the first to the third day of treatment. The parasites may disappear as early as on the 2nd day or as late as on the 7th day of treatment. Sinton (1928) found that 30 per cent of patients suffering from benign tertian infections relapsed after intermittent treatment with plasmoquin and 23 per cent relapsed after ordinary treatment with quinine. Immediate results obtained in acute attacks after treatment with this drug were however no better than those obtained with cinchona alkaloids. In quartan fever similar results were obtained but the parasites remained somewhat longer in the peripheral blood. Although the number of cases was small, no relapses were reported in quartan fever. Plasmoquin and quinine together, are believed by many authorities to be more effective in destroying the *sexual* forms of benign tertian and quartan parasites, than quinine alone.

M.T. infections.

As regards subtertian infections, plasmoquin has no action at all upon the schizogony cycle, and the parasites multiply unchecked. On the other hand it has the unique and remarkable property of completely destroying the gametocytes or 'crescents',—a property which is not possessed by either quinine or atebrin. This effect on the crescents can be studied microscopically; on the first day after plasmoquin administration the crescents are seen to be degenerated and stain badly; on the second day they are so degenerated as to be hardly recognisable; on the third day none are found. None of the cinchona alkaloids have any effect on the crescents nor do the arsenicals or the aniline dyes affect them adversely but even small doses (0.02 to 0.04 gm) of plasmoquin daily for three days are said to render the peripheral blood non-infective to anopheline mosquitoes; a single dose may render crescent containing blood non-infective for three days. The devitalising action is said to become apparent two days after the administration of the first dose. These facts are of great importance from the point of view of the transmission of malaria from one person to another. The property of destroying the gametocytes of malignant tertian infection holds out a hope for eradicating malaria by mass treatment. *Mollow* (1928) described the direct toxic effect of the drug on the crescents and the changes which take place in these bodies. Definite signs of degeneration were noted in crescents within 36 hours of the commencement of plasmoquin medication, and 24 hours later the crescents were entirely disintegrated. According to Fischer and Weise (1927) the daily dose of the drug, to remove the crescents from the blood, is 0.02 to 0.03 gm (1/3 to ½ gr.).

Crescents.

Practical trials of antigametocyte action of plasmoquin have been made by Clemesha (1933). Although the trials were on a small scale, the results show that it is possible to control the spread of malaria by careful antigametocyte work. Two doses of quino-plasmine a week usually effect a great reduction of parasites (trophozoites or schizonts) in the peripheral circulation. Malignant tertian and benign tertian gametocytes are never found if the dose is taken regularly. Quartan parasites are less acted upon by plasmoquin than other two varieties. Two doses of quino-plasmine a week cause the attack, even of malignant tertian, to be mild in character. The objection to this procedure is the trouble and expense involved; but the cost is much less than that occasioned by a severe outbreak of malaria.

Dosage.

The maximum dosage without producing toxic effects is 0.03 to 0.04 gm per day in Indian patients. According to Muhlens the dosage for quartan and tertian malaria is 6 plasmoquin compound tablets daily each containing 0.01 gm of plasmoquin and 0.125 gm of quinine divided into 2 or 3 doses. This means 0.06 gm of plasmoquin and 0.75 gm of quinine per day; 0.02 gm may be given 3 times a day for a week. The fever disappears in 1 to 2 days and the parasites in 5 or 6 days. The amount of quinine to be given daily in combination with plasmoquin depends on clinical symptoms. In chronic cases with acute symptoms 1.0 gm may suffice. In acute cases it may be necessary to give larger doses. In ambulatory cases not under daily supervision not more than 0.04 gm of plasmoquin should be given daily for periods not exceeding six days. The patient usually does not develop tolerance to the drug. For therapeutic purposes, the Fourth General Report, League of Nations (1937) recommends for adults a daily dose of 0.01 to 0.03 gm, associated with quinine or atebrin given either simultaneously or after a lapse of time. Even with such small doses when given simultaneously with atebrin toxic reaction may occur.

Children.

Children tolerate plasmoquin very well and it has the advantage of being tasteless; the maximum dose in children up to 6 months of age is 0.0025 gm three times a day, 6 months to 2 years 0.005 gm, 2 to 4 years 0.0075 gm, 4 to 8 years 0.01 gm, 10 to 15 years .0.02 gm.

The dose is varied according to the physical condition of the child, giving about 0.001 gm per kilo body weight. Intramuscular injections using the same doses are required in exceptional cases only. Intravenous injections should be strictly avoided. The drug should never be given without medical supervision. The after treatment can be varied according to the desire of the physician.

Plasmoquin in pregnancy: In therapeutic doses plasmoquin has no effect on the uterine contractions and it is considered to be safer than quinine in malaria in pregnancy.

Plasmoquine-compound:—The insufficient action of plasmoquine on the ring forms and schizonts of subtertian malaria led to the use of a combination of plasmoquine and quinine sulphate given in tablets, each containing 0.01 gm and 0.125 gm respectively. In this proportion the toxic effects of both the drugs are minimised. It is desirable that the dose of 0.06 gm of plasmoquine per day should not be exceeded, as otherwise toxic symptoms are produced. A much smaller amount than this may effect a cure and the presence of small quantities of quinine, which otherwise would not be effective gives better results when combined with plasmoquine.

In subtertian malaria treated with plasmoquine compound, the fever usually disappears after 2 to 3 days, and the parasites between the 2nd and the 7th day. Relapses do occur but they are not so frequent as with quinine alone.

Quinoplasmine or chinoplasmine is a combination with larger proportion of quinine than in plasmoquine compound. Each tablet contains 0.01 gm plasmoquine and 0.3 gm (4½ gr) quinine; the dose is three tablets daily for adults.

Cartain strains appear to be resistant to plasmoquine since in some areas only a few relapses occured after treatment with 0.03 gm of plasmoquine and 15 grains of quinine. Yet in other areas same treatment was followed by 50 per cent relapses (Malaria Commission of Holland 1932).

Manifold investigated the effects of quinine and plasmoquine when given simultaneously in European and Indian troops in India. He gave 0.04 gm of plasmoquine and 1.2 gm of quinine daily for 21 days in ordinary cases and for further five days in malignant tertian cases. He obtained excellent results and considers this combination to be a definite advance upon quinine alone for the prevention of relapses. However, since complications were noted in 0.1 per cent cases another preparation containing plasmoquin in still smaller amount has been produced and called quinoplasmin. It has been observed that quinine and plasmoquine when combined reinforce each others effect towards malaria and the untoward effects are minimised.

(3) Toxicity and Prophylactic Value

Toxic effects.

Like most of the potent synthetic remedies plasmoquine is a toxic drug. Even such small doses as 0.02 gm continued for five days produce such ill effects as asthma, cyanosis, vomiting and gastralgia in 50 per cent of cases. The appearance of untoward effects depends not only on the dosage given but on the treatment and susceptibility of the patient. Nervous patients are particularly liable to suffer. Cyanosis and epigastric pains were observed very early after trials in man, and they are universally present though they vary a great deal in their intensity in different individuals. Slight jaundice, cyanosis and abdominal pains are by no means rare. The appearance of cyanosis ought to serve as a warning to reduce the dose or to stop the drug. Cyanosis has appeared after 0.08 gm. In mild cases there may only be cyanosis of the fingers, nails, toes, ears and tongue, the face and the whole body may be involved. According to some it rapidly disappears on subsequent treatment with quinine, plasmoquine and quinine being mutually antagonistic in this respect. Abdominal pains and gastric disturbances are common when the drug is given on an empty stomach in large doses. Some believe they are due to rapid decrease in the size of the spleen. It is stated that these pains do not appear if the drug is given after meals.

Others have reported sudden attacks of pain which became very alarming and in many cases a course of treatment cannot be finished without toxic symptoms. Daily doses of 0.04 to 0.06 gm or even of 0.02 gm if prolonged for ten days may produce toxic symptoms.

Toxic symptoms as a rule have not been recorded unless the dose reaches 0.18 gm. The face is livid grey, but there is no dyspnoea nor undue distress. The patient complains of headache, dizziness, generalised urticaria, trembling, curious clammy sweats, abdominal pains, vomiting, and a sensation of bruising

over the lower ribs. The pain may be specially marked in the region of the liver, and central necrosis of liver lobules has been observed. In some cases the symptoms resemble an attack of cholera,—subnormal temperature, vomiting, diarrhœa and cyanosis being present. Cyanosis may be associated with hæmoglobinuria, involvement of the liver and intestinal symptoms.

The kidneys may show evidence of acute hæmorrhagic nephritis and the liver of parenchymatous necrosis such as is associated with the quinoline nucleus in such other compounds as cincophen. The blood turns chocolate coloured and methaemoglobinaemia and methaemoglobinuria occur. Urobilinogen appears in the urine simultaneously with the administration of the drug and persists for several days. The patient turns drowsy and becomes comatose. Pallor, with a marked drop of erythrocytes and haemoglobin count, exhaustion, and yellowness of the skin, are some of the symptoms. Haemoglobin falls to 30 per cent the leucocyte count rises to 24,000 per cmm, and the oxygen capacity of the blood is decreased. Methaemoglobin may appear in the urine 24 hours after cyanosis and may be accompanied by albumin and casts. The picture resembled that of mild blackwater fever. Jaundice may occur. Cyanosis may last for 24 hours after the drug is stopped but rarely may last as long as seven days. The serum becomes brownish with a strong direct bilirubin reaction and with urobilin strongly positive. There is a good deal of destruction of red corpuscles, the number falling to half the normal amount.

Methaemoglobin formation and cyanosis.

Many explanations have been given for the occurrence of cyanosis. It has been said to be due to disturbance of the heart rhythm, but most authorities believe it to be due to the formation of methaemoglobin, which has been detected in the urine and in the serum.

It is more likely to occur in patients with chronic constipation. In vitro plasmoquine mixed with 1 in 50,000 of blood produces methaemoglobin.

There is no direct connection between the amount of the drug given and the quantitative degree of change in the blood, although the most violent symptoms are only seen after large doses. The number of erythrocytes present in the blood appears to have a great influence on the toxic symptoms. The greater the anaemia, the quicker and more violently methaemoglobin is formed, and the deeper is the cyanosis. It follows therefore that patients suffering from secondary anaemia should be given only small doses of plasmoquine. The temperature of the individual is also a determining factor, the higher the temperature the more quickly is methaemoglobin formed. In certain patients cyanosis occurs even with small doses of the drug. The quinoline chain apparently is not responsible for the formation of methaemoglobin, but probably the alkamino group is really responsible and this occurs in such methaemoglobin-forming drugs as acetanilide and phenacetin. Cyanosis generally appears on the 5th or 6th day of the treatment.

A direct haemolytic effect on the part of this drug is disclaimed and it is believed that it acts on the reticulo-endothelial cells (Kupfer's star-cells) of the liver, whereby its detoxicating properties are decreased.

On account of the frequency of toxic symptoms, which are sometimes of an alarming nature, plasmoquin should only be used under hospital conditions. The drug is not as suitable for mass treatment as is quinine.

Prevention and treatment of toxic effects. The poisoning can be prevented by careful dosage, taking into consideration the body weight and general condition of the patient. A few drachms of glucose given daily, either in powder or liquid form, may prevent the onset of toxic symptoms. The drug should be given an hour and a half after a light meal. The bowels should be kept open. When symptoms have supervened the drug should be stopped and the patient strictly confined to bed and given copious alkaline drinks. Stimulants and mild non-irritating diuretics are indicated. In severe cases of methaemoglobinaemia and methaemoglobinuria the patient may be benefited by transfusion of blood.

Prophylactic value. Owing to the action of plasmoquine on the gametocytes, the question of its utility in malaria epidemics is worth considering. Experiments carried out in Java showed that plasmoquine had a well marked prophylactic value, especially in benign tertian malaria. *Use in epidemics.*

Mode of action of plasmoquine. The exact manner in which plasmoquine produces its destructive action on the malarial parasites is not understood. It has been suggested that the action of plasmoquine in malaria may be at least partly due to its capacity of converting haemoglobin into methaemoglobin. This would lead to the lysis of the red blood cell and consequent destruction of the parasite. According to some authorities the action of plasmoquine on malarial parasites appears to be direct, since morphological changes are readily seen microscopically in the gametocytes of *P. falciparum* as well as in those in birds. *Mode of action.*

There is evidence to show that substances, such as glucose which play a part in cell respiration, increase the action of plasmoquine; on the other hand the action of quinine is unaltered by the addition of such respiratory metabolic products.

Plasmoquine in blackwater fever:—The question whether plasmoquine is liable to induce blackwater fever is as yet unsettled. The observations of Amy and Boyd (1936) in India suggest that in certain regions it may be responsible for an increase in the number of patients with hæmoglobinuria. It is possible also that prolonged use of plasmoquine may interfere with the new formation of hæmoglobin. *Haemoglobinuria.*

Conclusions.:—Plasmoquine is a toxic drug, and the hope that it will replace the cinchona alkaloids in the treatment of malarial fever has not been fulfilled. On account of its toxic effects it has to be given under strict medical supervision. The action in rendering gametocytes non-infective to mosquitoes is of very great importance, and with plasmoquine compound there is possibility of sterilising the carriers. For the treatment of benign tertian infections the combination with quinine (plasmoquin-compound) may be suitable as it is found that a smaller dose is quite sufficient if supplemented with quinine. Although it acts on the asexual phase of benign tertian and quartan forms of malaria, it has no effect on the asexual cycle in malignant tertian malaria. For this reason and on account of its toxicity this drug should not be used in the treatment of malarial paroxysms. Its chief use is to destroy gametocyte of malignant tertian when they are present in the blood. For this purpose such small doses as 0.01 gm twice daily for 2 or 3 days are quite effective. Such doses do not, as a rule, produce toxic effects and according to some as much as 0.06 gm daily may be given without untowards effect. *Summary.*

Cilional was introduced by Schulemann and belongs to the plasmochin series of preparations; its action on gametocytes of *Plasmodium falciparum* was claimed to be as potent as that of plasmochin, in doses far less than any that produced toxic symptoms. A total dosage of 0.35 to 0.4 gm of cilional, administered in doses of 0.03 gm three times a day, is usually sufficient to eradicate practically all gametocytes of *P. falciparum* from the blood and even this high dose has been borne by patients without any ill effects. Missiroli and Mosna (1938) treated a series of *P. falciparum* gametocyte carriers with varying doses of cilional and concluded that in respect of its toxicity the drug is far superior to plasmochin and that addition of such a drug to quinine or atebrin will give a complete anti-malarial remedy. The writer (1938), however, carried out trials with this drug and considers that plasmochin is preferable to cilional, in so far as much smaller and practically non-toxic doses of the former drug effect eradication of crescents in a comparatively shorter time; further plasmochin is cheaper than cilional. *Cilional.*

Certuna is dialkylamino-oxyquinolamino-butane and is also said to possess gametocidal properties very similar to plasmoquine. The dosage recommended is 0.01 to 0.02 gm thrice daily for 3 to 5 days. It can be used in combination with atebrin. *Certuna.*

Plasmoquine is issued in tablets in three varieties:— *Preparations*

Plasmoquine simplex 0.01 and 0.02 gm. Dose 1 to 3 tablets daily.

Plasmoquine compound containing plasmoquine 0.01 gm and quinine 0.125 gm.

Quinoplasmine or *quino-plasmoquin* containing plasmoquine 0.01 gm and quinine 0.3 gm.

Preparations:—Pamaquin B.P. is a yellow to orange powder; odourless; taste, bitter. Insoluble in water. Dose 10 to 20 mgm or 1/6 to 1/3 gr.

10. Mepacrine (Atebrin)

Introductory.

This product was first called 'Erion' or plasmoquin E or Atabrine in U.S.A., is the dihydrochloride of 2-chloro-5-(diethylamino-methylbutylamino)-7-methoxyacridine. Quinacrine hydrochloride is the U.S.P. equivalent. It differs from plasmoquine only in the nucleus structure which is 2-chloro-methoxy acridine in case of atebrin and 6-methoxy-quinoline in case of plasmochin. The side chain is same in both. Compounds of similar composition have been produced in different countries and named differently *e.g.*, quinacrine, a French product, crinorin an Italian product and acriquine, a Soviet Republics product are all similar as regards chemical constitutional formula.

Atebrin is a yellow powder having a bitter taste. Its solubility which is only 2 or 3 per cent at room temperature increases to 7 per cent at 40°C. Like quinine, it is flourescent. Atebrin when dissolved in water hydrolyses slowly into freely soluble and sparingly soluble compound so that atebrin in solution is unstable.

(1) Pharmacology

Administered by the mouth atebrin is quickly absorbed from the small intestines and passes into the blood stream whence it is slowly excreted unchanged by the kidneys and the biliary tract. Beyond a slight irritant action on the gastric mucosa there are no effects referable to the gastro-intestinal canal. It is fairly well tolerated but when large doses are administered in animals, diarrhoea and reflex salivation occur; frequent vomiting is also noticed; the diarrhoea, however, usually subsides within 24 hours. If the drug is injected intravenously into rabbits under anaesthesia, a fall of blood pressure is obtained within 1-2 minutes. This fall is not due to any direct action on the cardiac musculature, but is probably due to a transient dilatation of the capillaries in the splanchnic and peripheral areas. Slight fall of blood pressure in man has been noted by Ganguli (1933) in a few cases. The fall of blood pressure has also been recorded in monkeys following an intravenous injection of atebrin. Nacht (1931) also obtained similar results in cats following intravenous injection of 2 mgm per kilo body weight. This fall of blood pressure appears to be due to action on vasomotor centres. According to Chin (1937) the excised heart of a rabbit is rapidly paralysed by direct toxic action on the heart muscle. The plain muscles of the small and large intestines and the uterus are not affected in any way after intravenous injections in anaesthetised animals. Whereas small doses stimulate both the pregnant and non-pregnant uterus, large doses may first stimulate but ultimately paralyse the uterus. If the liver is poor in glycogen due to malnutrition, urobilinuria may follow the administration of atebrin but this does not happen in normal healthy individuals. A slight reduction of temperature ranging up to 1°C has been obtained in rabbits with induced fever. This reduction in temperature lasts for about 12 hours.

Fall in Blood pressure.

Musculature.

Urobilinuria and Temperature.

Fate in the body and excretion:—Although it may not be possible to detect atebrin in the blood after twenty-four hours of its administration, it remains in the body for a considerably long period (up to 40 to 60 days). According to some a part of atebrin absorbed from the intestines is accumulated in the liver and spleen. The writer's observations on Indian patients confirm the view that there is some tendency towards accumulation of the drug in the body. Atebrin undoubtedly persists in the body for a much longer period than quinine or plasmoquine. If excretion is hindered there is a tendency for the appearance of the dye in the skin, which assumes a yellowish tinge.

Accumulation in organs

According to Hecht, atebrin is taken up by the organs which it meets first; thus for example, if atebrin be administered intravenously it will largely be taken up by the lungs which retain it longest, and in case of oral administration the liver will take it up. Atebrin is taken up by the liver, lungs, suprarenals, kidney and spleen mainly in that order and then follow stomach and intestines, heart, pancreas and genital organs. It is found in bile, saliva, semen, and hair. The muscles, skin and central nervous system take up atebrin but slightly. Atebrin is rapidly taken up by the tissues from the blood. Following an intramuscular injection, the level of atebrin can be determined in the blood after an hour, but this is not possible when atebrin is given orally. Mepacrine passes the placental membrane

and circulates in the foetal blood. Concentration in red blood cells and plasma is the same.

Excretion.

Atebrin is eliminated slowly and may be found in the urine many days after completion of a course. The urine should be examined daily during treatment, as its absence indicates possible accumulation of the drug in the system. The drug appears in the urine on the second day after administration, though traces may occur within an hour and can be detected up to 15 to 20 days or even longer. The excretion is not regular and may stop for a day or two and then reappear. The urine is bright yellow in colour. The drug is also found in the fæces.

Quantitative estimation.

All methods described for the quantitative estimation of atebrin in the urine are based on the same principle. In general they all consist of the following steps: an immiscible solvent is used for extraction of urine; solvent is then evaporated and the residue thus obtained is dissolved in an acid, or instead of evaporating the solvent, the residue is extracted by an acid. The colour or flourescence of the resulting solution is then compared with appropriate standards. Various modifications have been reported.

Toxic effects.

Toxicity:—Cats tolerate oral doses of 0.05 gm per kilo body weight without any reaction. After administering 0.07 gm per kilo the animals show symptoms of gastro-intestinal irritation. On an average 0.1 gm per kilo by the mouth is the minimum lethal dose, the animals dying with symptoms of collapse. If large doses are administered, fatty degeneration of the liver and kidneys occurs. Rabbits tolerate the same dose without any demonstratable injury whatever. Blood changes, especially formation of methaemoglobin, have never been observed. A dose of 0.1 gm per kilo injected subcutaneously causes death.

In volunteers 0.6 to 1.0 gm of mepacrine produced headache often severe nausea, sometime repeated vomiting, colicky abdominal pains and diarrhoea; and in more severe cases fever and prostration. The symptoms began to disappear after 8-9 hours. X-ray examination with opaque meal showed increased peristalsis and increased secretion of stomach and colon. In persons taking 0.1 gm daily nothing abnormal was observed. Rarely, 0.2 gm twice weekly may produce diarrhœa and vomiting.

A case is recorded of a European in West Africa who was on mepacrine suppression treatment for 16 months. In a fit of depression he swallowed 250 tablets (25 gm) of mepacrine. Ten minutes later he vomited 10 to 14 times and became very weak and drowsy, and was cold, collapsed, and in a stuperose condition but could just be roused. The stomach was washed. He was drowsy, resented being moved and could not answer questions; pupils were contracted and reacted to light. An intravenous drip of 30 per cent of glucose was given with 5 mgm of riboflavin (in view of its antagonistic action to mepacrine); 2 pints were given in 4 hours. Patient recovered the following morning.

Daily administration of 0.1 gm of mepacrine for a period up to 12 months in women produced no symptoms of any consequence, though mild gastro-intestinal symptoms occured in a few. General health, weight, pulse rate, blood picture, menstruation, and hair remained unaffected. Yellowing occured primarily in the pigmented areas of the skin. Mepacrine plasma level of 25 mgm/litre was maintained on a daily dose of 0.1 gm.

Therapeutic index.

Chemotherapeutic studies. The therapeutic efficacy of the drug was evaluated in bird malaria by Roehl. He worked out a method of using canaries for experiments on lines closely approaching the conditions of practical therapy so that it was possible to try out and assess in the laboratory many groups of drugs. Here it was shown that in bird malaria, the therapeutic index of atebrin was 1: 30. Plasmoquine has the same therapeutic index whereas quinine has an index of only 1: 4. Atebrin therefore is four times as effective as quinine. Chopra and Das Gupta (1933) carried out elaborate studies on the action of atebrin on plasmodium infections in monkeys and came to the following conclusions.

Monkey malaria.

Action on the plasmodium. The destructive action of atebrin on this plasmodium appears to be powerful. Usually two doses of 0.025 gm each given either intramuscularly or intravenously are sufficient to control a very heavy infection which may amount to a million parasites per cmm. The drug equally effects both the schizogony and the gametogony of the plasmodium and all phases of the parasite disappear rapidly from the peripheral circulation under its action. Even after a single dose, signs of degeneration are seen in the parasites and their numbers rapidly decrease. After two or three doses they disappear from the peripheral blood altogether.

Owing to its slow excretion, atebrin, when given intravenously, appears to exert a more prolonged action than quinine on *P. knowlesi.* Intravenous injections of quinine are not

effective against a heavy infection with this plasmodium in *M. rhesus* unless they are repeated at short intervals, say of three or four hours, but one injection of atebrin suffices. It would appear that the growth of the parasites is not checked by quinine, probably because of its rapid excretion after intravenous injection.

In monkey malaria relapses undoubtedly occur. In *M. rhesus* infected with *P. knowlesi* even after 5 days intensive treatment with large doses of the drug, the parasites invariably reappeared in 10 to 15 days and multiplied with the same rapidity as in the primary attack, causing death of the animal if prompt treatment was not given. The recrudescence could however be checked much more easily than the original attack. One dose as a rule sufficed to control the multiplication of the parasites though a low grade of infection persisted for a long period. After such a dose the parasites appear to loose their virulence and either a scanty infection may persist or the parasites may disappear from the peripheral circulation for long periods without further treatment.

(2) Effectiveness in Malaria

The antimalarial efficiency of the drug was first tested by Prof. Sioli in syphilitic paralytics artificially infected with benign tertian malaria. He administered the preparation to patients on 3 consecutive days and found that 0.1 gm three times a day could effectively control the symptoms and cause a disappearance of the parasites.

Indian strains of malaria.

Chopra and his co-workers (1933) tested this drug on the Indian strains of malaria and have come to the following conclusions:—

(1) Atebrin is an effective drug in the treatment of Indian strains of malaria. Its destructive action on the asexual forms of benign tertian, malignant tertian, and quartan types of malaria is about equal, the schizonts disappearing from the peripheral circulation after 0.6 to 0.9 gm of the drug, *i.e.*, the administration of 3 tablets of 0.1 gm for 2 or 6 days.

(2) The sexual forms or gametocytes are more slowly acted upon than the asexual forms. The gametocytes of the benign tertian and quartan types are destroyed and degenerative changes can be observed in them shortly after the administration of the drug is started. The gametocytes of the malignant tertian type, *i.e.*, crescents, are not affected at all.

(3) The drug is effective in doses of 0.1 gm three times a day, the course lasting for five days, making a total of 1.5 gm of the drug for the cure. In the majority of patients such a course is effective, but in a few of the persistent ones it may have to be repeated after a few days interval. The drug can also be effectively given intravenously in doses of 0.1 gm dissolved in 1 to 2 cubic centimetres of distilled water when the number of parasites in the peripheral blood is large.

(4) In chronic types of malaria the drug is effective and produces a rapid reduction in the size of the spleen.

(5) Atebrin is reported to prevent relapses, but the evidence at our disposal shows that this is not the case with Indian strains of malaria. Its prophylactic value is very similar to that of the cinchona alkaloids.

(6) The blood pressure is lowered in some patients during the administration of the drug, but in the majority it has no effect. The pulse rate and respiration are not markedly affected. It has been used in patients suffering from endocarditis and myocarditis without ill-effects.

(7) The action of atebrin closely resembles that of the cinchona alkaloids

and the introduction of this drug is a distinct advance in the treatment of malarial fevers in India.

Action on Sexual forms.

Observations made at Horton by Sinton have shown that atebrin is not without effect upon the crescents of the Roumanian strains of *P. falciparum* used in that country for malaria therapy. If the drug is administered before these forms are detected in the peripheral blood, it appears to cause morphological changes in the immature stages of the sexual forms.

The effects of atebrin continue for a longer period after the cessation of treatment, the decrease in the percentage of enlarged spleens continues longer, and the return of splenic index figures to their former level occurs more slowly than when quinine is employed. The exact effect of atebrin on the process of immunisation requires further study, as do the toxic results of prolonged administration. There are instances of patients who have taken daily therapeutic doses of atebrin for as long as two and a half years without toxic results. Treatment with this drug is believed by some to be less effective in benign tertian and quartan than in subterian malaria.

Relapses.

It was said that the most valuable property of atebrin is its power to prevent relapses, and many workers have borne testimony to this effect. In the author's experience, a large number of patients relapsed. Although in an endemic area it is difficult to prove in the human patients that relapses actually did occur after a course of atebrin, there is ample evidence in experimental malaria in monkeys (*M. rhesus*) that the drug does not eradicate infection from the body and that relapses are common. In quite a number of these animals, after a course of the drug, and disappearance of symptoms and parasites from the peripheral circulation, the parasites reappeared usually within two weeks and the animal showed symptoms of the disease.

Combination with plasmochin.

According to Chopra and others, in cases of benign tertian malaria the combination of atebrin-plasmochin is no more effective than atebrin alone, as judged by the rapidity of the disappearance of parasites from the blood stream. In malignant tertian cases however, it appears to be more effective, and relapses are less frequent when the combined drugs are administered. Both methods of treatment are of equal value with regard to reduction in the size of the spleen. The combination of the two drugs is more toxic than is atebrin alone, and is not therefore recommended for ordinary cases. The combination has distinct advantages in the treatment of malignant tertian cases, especially if crescents are present, but has no particular advantages in the treatment of benign tertian and quartan cases. The best way is to give the usual course of atebrin and follow it up with 0.01 gm. of plasmoquine twice daily for 3 to 5 days.

Combination with quinine.

Atebrin in combination with quinine, 7 to 10 grains daily, has been used in severe cases of malignant tertian malaria. The symptoms are said to be more rapidly controlled than when the drugs are given individually and the relapse rate is also less. This is doubtful.

Comparative action with quinine.

The two drugs are equally effective against malaria, but the effective concentration in the blood is more rapidly attained in case of quinine than with atebrin; its (quinine's) duration is shorter on account of its oxidation in tissues and rapid excretion. If a large initial dose of atebrin is given the effective concentration in the blood is more quickly reached than with small doses.

Dosage. Atebrin is usually given by the mouth, the tablets (0.1 gm) being taken on a full stomach and washed down with plenty of liquid.

Course of treatment.

The average total daily dose for an adult is 0.3 gm taken in three equal amounts. Some physicians prefer to give the drug in one dose, especially when

large numbers of patients are being treated; very heavy persons over thirteen stone are given a total of 0.4 gm daily in four equal doses.

Children, even unweaned babies, tolerate atebrin well, and the following daily doses are recommended:

Up to 1 year 0.05 gm (*i.e.* ½ tablet), 1 to 4 years 0.1 gm, 5 to 8 years 0.2 gm, Over 8 years 0.3 gm. Atebrin is best taken with milk or water. The above mentioned doses are based on very wide experience; however, weight and general condition of patients should be taken into account.

Regardless of the patients temperature, administration of atebrin is begun immediately and continued uninterruptedly for seven days. The five days course originally recommended seems too short, especially in malignant tertian malaria, in which at this point isolated parasites still occasionally appear. On the other hand, experience in India shows that an extension or repetition of treatment is often unnecessary. It may be mentioned here that some authorities continue treatment for ten days, and recommend a repitition of this treatment after an interval of seven days.

Parenteral administration.

Perenteral routes—(*a*) *Intravenously* 0.1 gm of atebrin is dissolved in 3 ccm of distilled water and as much as 0.3 gm in 9 ccm of water can be injected, generally without untoward effects. Only after 0.4 gm had been given, are serious symptoms such as collapse, observed. In cerebral malaria, with doses of 0.1 gm to 0.2 gm in comatose cases in Ceylon, good result were obtained. A proprietary preparation named atebrin-musonate (atebrin dimethane sulphonate), 0.375 gm, which corresponds to 0.3 gm of atebrin dichlorhydrate (*i.e.* ordinary atebrin), has been given by the intravenous and intramuscular routes. Atebrin-musonate is marketed in ampoules containing the equavalent 0.1 and 0.3 gm of atebrin, and the contents are dissolved in 3 or 9 ccm of distilled water respectively before administration. The dose of the latter in children under 12 year is 1 to 5 ccm, and for adults 6 to 9 ccm. The drug appears to be effective almost as quickly if administered intramuscularly as by the intravenous route.

Intravenous route.

Intramuscular route.

(*b*) Intramuscular injection is suitable not only in comatose malaria, but also in cases with gastrointestinal disturbances, and for children. It is less painful than quinine. During widespread epidemics this method may be useful for mass treatment; although two injections (after an interval of 24 hours) have given excellent immediate results, the treatment must be continued by the mouth in order to prevent relapse. Though the immediate effects of intramuscular and intravenous routes are rapid, the cure is not permanent and relapses are frequent. Sterile abscesses may form. The parenteral route should not be used when severe anaemia is present. In doses of 0.2 gm it has been given in cerebral malaria by the intravenous route but it is believed to be not as effective as quinine.

Prophylactic value.

Atebrin is claimed to have prophylactic properties and has been used for this purpose with good results. It has been continued for months in doses of 0.1 gm daily without producing any untoward effects. It has also been combined with plasmoquine for this purpose, 0.1 gm of atebrin and plasmoquine 0.01 gm being given together daily, but this has no particular advantage. Atebrin, however is not a casual prophylactic drug and its value is entirely of suppressive nature.

Suppressive Therapy.

Suppressive action. Neither atebrin nor quinine act as true causal prophylactic drugs in malaria, but when atebrin is administered in proper doses it suppresses the

clinical symptoms and prevents paroxysms. Malarial infections are thus held at subclinical levels. Such use of the drug is specially valuable for military forces operating in endemic areas, and was extensively employed in World War II. The method usually adopted is to give 0.1 gm daily with the morning or evening meal making a total of 0.7 gm per week. Others give larger doses 1.0 gm per week, because with 0.1 gm daily adequate concentration in the blood is not reached till after the third week and the drug has therefore to be started two weeks before the expected period of exposure. The administration of the drug should also be carried on for sometime after leaving the malarial area. Usually cases of clinical malaria occur 10 days to three weeks after the drug is stopped. In case of intolerance to atebrin, quinine in doses of 0.5 gm (7½ grs.) daily may be used. Another method of chemoprophylaxis is to give 0.2 gm of atebrin twice a week and this has also proved effective in military practice during World War II.

Atebrin 0.1 gm was given daily to a series of 43 healthy men for periods of 9 to 12 months without any impaired efficiency of the liver and kidney, as shown by function tests. In another series of 95 cases 0.4 to 0.7 gm weekly were given for 4 to 10 months, there was no significant change in red or white blood corpuscles, haemoglobin, or sedimentation rate.

The action is upon the schizogonic forms of the parasite in red blood corpuscles and not upon the sporozoites. The sporozoites of *P. falciparum* in the salivary glands of anophelines are able to withstand a concentration of 1 in 2,500 atebrin for thirty minutes, since they are afterwards able to cause infection in susceptible human subjects.

So far as clinical and mass prophylaxis is concerned there is, however, evidence that biweekly doses of 0.2 gm of atebrin administered during the whole of the malaria transmission season and even for a few weeks longer exert a definite action on the number of malarial attacks. This method was tried on a large scale in the army during World War II with good results and no toxic effects were observed.

Direct action.

Mode of Action:—The evidence, which however is not extensive, suggests that the action of atebrin on the trophozoites of malaria parasites is direct. James (1934) showed that after a single dose of 0.6 gm of atebrin, morphological changes rapidly occur in the parasites of benign tertian and quartan malaria. The pigment becomes aggregated into clumps and disappears, while the cytoplasm is ragged and also eventually disappears: the nuclear vacuole is distended, the chromatin opens up and becomes diffuse till only a few lightly stained dots remain. Similar changes have been noted by Chopra and coworkers (1936) as a result of studies on the action of atebrin on *P. Knowlesi*, and they also made the important observation that a number of flourescent dyes, including atebrin, are fixed by the plasmodia of bird malaria.

(3) Toxic Effects

Toxicity.

Atebrin unlike plasmoquin is not a very toxic drug. Double the usual dose of 0.3 gm per day (*i.e.*, 0.6 gm in 24 hours) can be tolerated, but larger doses may produce gastro-intestinal irritation. In spite of the tendency referred to above of accumulation of the drug in the system, the writer gave two five day courses of the drug with a few days interval between without producing any toxic effects, and with therapeutic benefit. Some patients complained of slight pain or a sensation of uneasiness in the epigastric region soon after taking the drug. This generally started on the second or third day and persisted as long as the drug was being administered. The pain was never so severe as that produced by plasmoquine. In none of the series were the severe abdominal pains described by Green (1932) observed. A number of patients complained of headache and loss of appetite while the drug was being given, but this also passed off when atebrin was stopped. In some of the patients, diarrhœa was produced on the second and third days of treatment and persisted while the drug was

being given. The diarrhœa was of a mild type and no particular treatment was necessary. It stopped with the cessation of the drug. In a few patients, especially those who were obese, palpitation occurred but it stopped when the drug was discontinued. A yellow staining of the skin and conjunctiva occurred in several of our patients, but the colouration as a rule was very slight and in none of the patients did it amount to a jaundice-like appearance. It is not true jaundice but merely represents excretion of the drug by the skin. Some workers consider that intense pigmentation of the skin is necessary to produce a cure.

Pigmentation.

Atebrin pigmentation is most marked on the dorsum of the hands and feet and also on the face and forehead. The sclerae are not markedly tinted. Atebrin pigmentation may be mistaken for other conditions which cause mild jaundice, such as pernicious anaemia, yellow fever, subacute bacterial endocarditis, and Addison's Disease. The urine should be examined for atebrin for diagnosis. Urinary and intestinal excretion should be kept at high level during atebrin treatment. Saline purgative should be given and large amounts of fluid should be taken while atebrin is being administered.

Mental disturbances.

Mental disturbances have been observed in patients who have received atebrin. In some, the drug has acted as a cerebral excitant, the patient experiencing a feeling of undue excitement, a sense of exhilaration and well being; a mild or transient psychosis was observed in others, while in rare cases serious mental symptoms accrued. It has been suggested that this may be the result of liberation of malarial toxins which act on the cortical centres. Others believe that it represents the emergence of pre-existing personality defects and not so much the specific toxic action of the drug.

To prevent reactions, the atebrin should be taken after the evening meal or with a meal after which there is a long period of physical rest. Plenty of sugar or glucose with the meal is also helpful. If a patient shows signs of intolerance, start with a small dose and then increase gradually.

Preparations:—

(1) Mepacrine hydrochloride B.P. is a bright yellow crystalline powder; odourless; taste bitter; solubility in about 40 parts of water. Dose 0.1 gm or 1½ gr. Therapeutic doses—0.2 to 0.5 gm or 3 to 8 gr daily in divided doses in tablet form.

(2) Mepacrine methanosulphonate, a bright yellow, crystalline solid; odourless; taste bitter; soluble in 3 parts of water and 3 parts of alcohol. Dose—0.1 to 0.3 gm or 1½ to 5 gr.

11. Other Drugs Used in Malaria

Hydroquinine or methylhydrocupreine, is obtained by the hydrogenation of quinine. A combination of this compound with an acridine dye and a bile salt has been introduced under the name of Tebetren, which is described as methyl-hydrocupreine-methyl-acridine-dehydrocholate.

Tebetren.

The bile salt is said to render the compound less toxic. Malarcan is a preparation of similar nature prepared by an Austrian firm. The drug is available for oral administration in tablets of 3 grains each and in ampoules of 2.1 ccm and 1.1 ccm for perenteral administration.

Malarcan.

Chopra and his co-workers tested malarcan in a series of cases. The drug controlled the clinical symptoms of malaria in much the same way as quinine, but parasites took longer to disappear and relapses were more common. It has no effect on the sexual forms of malignant tertian parasites.

The Italian Biochemical Institute, Milan, prepared a drug 'M_3', which, according to the manufacturers, is composed of a mercuric manganese iodide and of concentrated extract of spleen. A number of experiments were conducted by the writer to verify the claims put forward by the manufacturers that it acts directly on the sporozoites inoculated by the anopheline mosquitoes and that for this reason its prophylactic action is almost absolute. It failed to prevent the development of sporozoites of Indian strains of *P. falciparum* in the human host, when infected mosquitoes were fed during a course of treatment by this drug and at varying intervals after the full course had been completed. *Ms*

Under the name of 'Paludex', a compound described as "copper oxyquinoline sulphonate of soda" was introduced for the treatment of malaria. Paludex is a greenish amorphous powder freely soluble in water with a neutral reaction, it is stable under ordinary conditions of temperature and humidity. The drug is said to have some activity against malaria in man in doses of 1 to 2 gm daily. In bird malaria, halteridium infections, and monkey malaria, no favourable action could be demonstrated.

Sulphonamide Compounds

For some years sulphonamide derivatives have been the subject of intensive study on the part of chemists, experimental pathologists, and clinicians. Prontosil was shown to be effective in the treatment of malaria by de Lein (1937), and Chopra et al. found that prontosil in doses of 3 to 4 gm daily for 5 days possessed an undoubted action in causing disappearance of malaria parasites (*P. falciparum and P. vivax*) from the peripheral blood, and controlling the symptoms of the disease. Chopra and Das Gupta (1939) tested the efficacy of M & B 693 in monkey malaria, and found that this compound is capable of destroying the monkey plasmodium (Plasmodium knowlesi) in doses which are even less than those regarded as the proportionate doses for a monkey as compared with man. Moreover, the parasites once disappearing after a five day's course of treatment do not appear again in the peripheral blood, at least in sufficient numbers to be detected in thick films. *Prontosil.* *M & B 693.*

Sulphapyridine, sulphathiazole and sulphadiazine are also effective, the action of the last named being the most powerful. On account of their toxicity, however, their use in the treatment of malaria is not recommended.

Miscellaneous Drugs used in Malaria

Certain other drugs are believed to have some influence in causing the parasites to disappear from the peripheral blood or perhaps in assisting the patient to resist the invasion of the parasites. These drugs can be divided into two groups: (1) those which are given in combination with quinine and (2) those which are given by themselves especially where quinine is not tolerated.

(1) Drugs used against malaria in combination with quinine

(*i*) *Preparations of Arsenic, Antimony, Iron, and Mercury.*

Various preparations of arsenic are frequently used in the treatment of malaria. Of the various *inorganic preparations,* liquor arsenicalis (Fowler's solution) and a combination of arsenic, quinine and iron, either in the form of a pill or a mixture have been prescribed. *Mistura arsenii quininae et ferri* (Baccelli's mixture) is one of these preparations and it is given in doses of ½ to 1 oz. (15 to 30 ccm). Arsenic in inorganic form has been shown to have no action whatever on the malarial parasites. *Arsenic.*

Organic preparations of arsenic are said to have a marked effect on some of the pathogenic protozoa and are believed by some to have a destructive effect on the malarial parasites. *Neo-arsphenamine* is said to abort the symptoms in practically every attack of malarial fever and thus save the patients from getting attacks of fever which are very debilitating. The convalescence is shortened and the chances of recovery are enhanced. They also have an excellent tonic value and the patients recover quickly and return to work. *Sulph-arsphenamine* in doses of 0.3 to 0.5 gm subcutaneously or intramuscularly is also effective.

Soamin and *arseno-benzol compounds, stovarsol* and *treparsol* have been frequently tried either alone or in combination with quinine. One gm of the sodium salt of stovarsol in 10 ccm of distilled water given intravenously cleared the blood of *P. vivax* in seven artificially infected and two naturally infected cases and prevented relapses for two months. *Soamin stovarsol.*

The drug acted better on old pigmented parasites, the older schizonts and the gametocytes. Sinton (1927) treated 25 cases of benign tertian infection with stovarsol giving up to 4.0 gm in 5 days; 92 per cent. relapsed. Stovarsol is said by some to be without effect on the parasites of quartan and malignant tertian fever.

Quiniostovarsol.

When it was found that arsenical preparations in general have only a feeble action on malaria parasites, it became the custom to combine these remedies with quinine. The mixtures chiefly used are *quiniostovarsol* (Fourneau) and *quinine troposan* (May and Baker). On behalf of the Malaria Commission several workers have made clinical trials with quiniostovarsol. The general trend of opinion seems to point to the fact that these drugs are not of much use.

Antimony and mercury compounds.

The view held by some observers that *tartar emetic or other compounds of antimony* have an effect on the malarial parasites has now been abandoned, though some of them still hold that they may be useful in preventing relapses.

Mercury compounds such as *perchloride, mercurophen, mercurosol* and *mercurochrome* 220 have been tried in the treatment of malaria without success. They have no curative action in human or avian malaria.

Methylene blue.

Coal tar preparations: The use of medicinal methylene blue (methylthionine hydrochloridum) against malaria was first suggested by Ehrlich and Gullaman. It has been tried by many observers with varying results. The preparation used should be free from metallic impurities, especially zinc chloride which may cause vesical and rectal irritation. The drug is given in doses of 1 to 4 grains (0.06 to 0.25 gm) in cachets or capsules 3 or 4 times a day for a week or more, the total daily dose not exceeding one gram. It is excreted in the urine which becomes blue and the stools also turn blue on exposure to air. In the pernicious type methylene blue has been given combined with quinine intravenously, 5 ccm of 1 per cent solution being injected. It has been tried in doses of 0.2 gm three times a day at two hour intervals, in the treatment of quartan malaria which does not yield to quinine so readily as the other forms. The writer has tried a combination of quinine with methylene blue in quartan infection and considers it superior to many other forms of treatment.

Carbolic acid preparations.

Liquid carbolic acid 6 to 12 minims daily for a period varying from 4 to 6 weeks have been tried in the treatment of benign tertian malaria but the results are disappointing.

Eusol.

Liquor hypochlorous co., which contains approximately 0.027 per cent of hypochlorous acid was tried intravenously in cases of malaria which appeared to be refractory to quinine. Quinine has also been combined with iodine, especially in the treatment of chronic malaria.

Quinine does not act efficiently when the liver is not active. For this purpose it has been combined with such drugs as calomel, rhubarb, aloes. Warburg's tincture contains aloes, rhubarb, saffron, gentian, ginger, cinnamon, camphor, pepper and a number of other things along with quinine sulphate. In doses of half an ounce 2 or 3 times a day on an empty stomach it is effective in the treatment of malaria.

Organotherapy.

Organotherapy has been used as an aid to quinine treatment. Preparations made from bone-marrow and suprarenal gland have been tried. Bone-marrow extract may be given in the form of tablets, 1 to 3 being given daily, or as a glycerin extract which is sold under the name of 'marrabain.' It has been suggested that low blood pressure in pernicious malaria is due to inefficiency of the adrenal glands and for this reason suprarenal gland extract has been given internally. Injections of adrenalin have also been used for constricting the blood vessels in certain organs and dilating them in others so as to flush them with blood containing quinine. Intravenous injection of adrenalin beginning with 0.01 mgm and gradually increased to 0.3 mgm have been given in the treatment of splenomegaly. As many as 40 injections may be required to reduce the size of the spleen. Dessicated spleen substance has also been used in doses of 3 to 10 grains daily. Pluriglandular extract, *i.e.*, a combination of suprarenal, spleen, pancreas and thyroid has been used on theoretical grounds. None of these produced any remarkable effect.

Radio-therapy.

Radio-therapy has been advocated especially in the splenomegaly of malaria, applications being made over the region of the spleen. In chronic cases the effect on the spleen is appreciable. In acute cases heavy dosage is dangerous. Taking into consideration the length of treatment, its difficulties and inconvenience, it should be rejected in favour of quinine and arsenic only.

(2) Miscellaneous Drugs used against malaria

A number of medicinal plants have been used in the treatment of ague in India for centuries, but none of them have shown any special effect on the malarial parasites. A brief description of some of these remedies which have been tested by modern scientific methods is given below:—

Vitex peduncularis. This plant grows largely in Bihar, Eastern Bengal and the Central Provinces but it is not very well known. The aboriginal tribes of these localities believe it to have curative properties against malaria, blackwater fever and kala-azar. In Hindi it is known by various names,—*Nagbail, Nagphani, Charaigora, Chhagriaruba, Minjurgorwa,* in Bengali it is called *Baruna* and *Goda.* *Vitex peduncularis.*

The only reference by the old writers regarding its medicinal properties is its use for external application for pains in the chest. Vaughan (1921) found that the aboriginal tribes of certain parts of Bihar were well acquainted with this plant, and used it in the treatment of malarial fever and also of blackwater fever. They prepare an infusion of the leaves, of the root-bark or young stems and take it internally several times a day with much benefit. Preference is given to the dark-coloured-root plant over the pale-coloured variety.

Vaughan (1921) tried this drug in a series of cases in both these diseases and reported that it gave very satisfactory results. Chopra, Knowles and others (1924) carefully tested the drug and found it has no effect what so ever in malaria.

Paganum harmala. Two alkaloids named *harmaline* and *harmine* occur in *Harmal* or *Peganum harmala,* which grows in abundance in Northern India, possess pharmacological action very similar to quinine. In acute malaria the action is not so pronounced as that of quinine, but harmine is said to have been successful in cases of relapsing malaria where quinine had failed. The writer has tried harmaline in doses of 5 to 10 grains daily without any effect.

Alstonia scholaris. Alkaloids named *ditamine* and *echitamine* are obtained from *Alstonia scholaris.* This plant grows all over India and is said to have strong anti-malarial properties. It has however been found ineffective. It does not strengthen the action of quinine when combined with it, nor does it reduce its effective dose as has been believed by some. *A. congolensis, A. macrophylla,* and *A. constricta* are rich in alkaloids and have all been tried. *Alstonia scholaris.*

Berberis aristata (Rasaut) which grows on the Nilgiris and in the Himalayas contain two alkaloids named *oxycanthine* and *berberine.* Both Alstonia and Berberis were largely used in India and the eastern colonies in the treatment of malarial fever. Waldrop (1927) advocated berberine as a provocative in latent malaria and considered it a specific in splenomegaly. The writer tried berberine sulphate in doses of 3 to 4 grains 2 to 3 times a day in the treatment of malaria without success. *Berberis aristata.*

Other drugs that have been used in the treatment of ague in the indigenous system in India are *Coesalpinia bonducella, Calotropis gigantea, Hydrocotyl asiatica, Melia azadirachta, Soymida febrifuga, Andrographis paniculata, Aconitum napellus* and *Picrorrhiza kurooa* but none of them have appreciable antimalarial properties.

Holarrhena antidysentrica. The bark is used in some part of India in the treatment of malaria. The writer has tried the alkaloid isolated from the bark, without effect. *Holarrhena antidysentrica.*

Opium and narcotine. Opium is said to assist the action of quinine but there appears to be no foundation for this belief, though it undoubtedly ameliorates the many disagreable symptoms of this disease. Roberts (1895) pointed out that the alkaloid *narcotine* or *anarcotine,* which occurs in large quantities in Indian opium possibly has antimalarial properties. Chopra and Mukherji (1930) tried the drug in a series of cases of all forms of malaria, in the Carmichael Hospital for Tropical Diseases, doses ranging from 6 to 12 grains daily and came to the conclusion that this alkaloid has no antimalarial properties whatsoever. Opium addicts taking such large doses of opium as 20 to 30 grains daily get attacks of malarial fever. *Opium and narcotine.*

A number of proprietary remedies have been recommended for the treatment of malaria but have found to be either useless or of little practical value.

12. The New Antimalarial Drugs

(1) Paludrine

The large amount of chemical synthetic work which had been carried out all over the world following the introduction of pamaquin (plasmochin) and mepacrine (atebrin), had been mainly concerned with derivatives of either acridine or quinoline, but no material advance had resulted. A number of attempts had been made by various workers to build up anti-malarial structures on other heterocyclic ring system, such as the phenanthridine, benzoquinolines, pyrimido-quinoline, quinazoline and carbazole rings, but little success attended these efforts. Mepacrine remained the outstanding substitute for quinine. With the lapse of time it became increasingly clear that no fresh advance was to be expected unless a completely new lead was obtained, and with this end in view investigations were commenced in the research laboratories of Imperial Chemical Industries Ltd., at Manchester by Curd, Davey and Rose.

(i) Experimental work

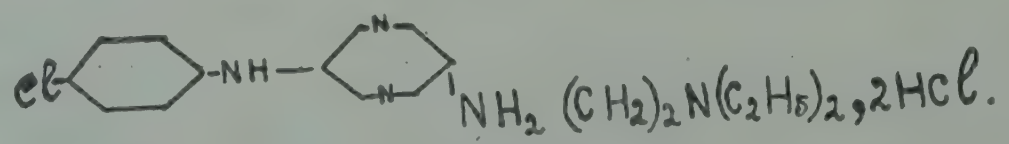
Chemical composition and anti-malarial activity.

The guiding principle in this work was the fundamental knowledge which emerged from the pioneer researches of Schulemann, Schonhofer and Wingler, and which led to the synthesis of pamaquin, namely that by linking a basic residue of a certain type of an amino group in a heterocyclic ring system, such as quinoline, acridine, etc., and in some cases in a non-heterocyclic structure, such as triphenylmethane, anti-malarial activity against avian malaria resulted.

The discovery of the anti-malarial action of sulphanilamide by Diaz de Leon in 1937 and its confirmation by increasing number of clinical reports, led to speculation as to their mode of action. As the sulphonamides show no structural resemblance to the other types of known anti-malarial compounds, it was argued that their activity might be due to the fact they are aniline derivatives of which the pharmacological properties are controlled by the sulphonamide group, which promotes absorption from the gastrointestinal tract and the penetration of the erythrocytes which are the habitat of the schizogonic forms of the malarial parasite. These properties appeared to be particularly suitable in the case of sulphadiazine and suggested that derivatives of pyrimidine without the SO_2 group might possess anti-malarial properties. Pyrimidine derivatives are of great physiological importance and take part in a number of fundamental life processes. It might, therefore, be anticipated that pyrimidine would possess important intrinsic chemotherapeutic properties, and, further, that these would include activity against the parasites of malaria.

It is postulated, however, that in order to produce an effective chemotherapeutic substance on the basis of an intrinsically active nucleus, it is necessary to attach to it certain pendant groupings, which may, among other functions, confer the correct pharmacological properties on the compound. In the case of mepacrine and pamaquin the important pendant grouping is the basic alkylamino group. The investigation of pyrimidine derivatives containing similar alkylamino groups was thus indicated as the first step.

Instead of starting with the simple pyrimidines, investigation of derivatives of the anilinopyrimidines was taken up, and here anti-malarial activity was immediately encountered in the compound 2666 which has the formula



2666. The compound 2666 was first prepared in October, 1942 and its biological activity against avian malaria was later established. This, however, was not of a high order and in clinical trials it proved inactive in human malaria. Modification of its chemical constitution led

3349. to the synthesis in July, 1943, of 3349, which showed marked activity against avian malaria, and later in clinical trials at the Liverpool School of Tropical Medicine its activity in all types of human malaria was demonstrated. The discovery of 3349 led to the synthesis

4430. of hundreds of new substances, some of which had powerful anti-malarial activity. 4430 proved to have considerable activity in all forms of malaria, but was superseded by

4888 or Paludrine. This drug was first synthesized in November, 1944, and clinical trials first at Manchester and later in Australia established its efficiency in all forms of human malaria. *4888. Paludrine.*

Two important considerations in the life-cycle of malarial parasites helped considerably in the discovery of these new compounds. Firstly Schaudinn observed that it has never been confirmed that injected sporozoites penetrate red blood cells directly, and secondly there is a period of days or weeks following the bite of a mosquito, before parasites can be demonstrated in the blood. The immediate fate of the injected sporozoites was until recently uncertain, but it has now been shown that a period of development of the parasite occurs in the tissues of the vertebrate, before the red cells are invaded, and it is the duration of this *'tissue phase'* which constitutes the incubation period. A simple experiment shows that the sporozoite quickly leaves the blood stream and that no blood infection can be demonstrated subsequently for several days. Thus if sporozoites of *Plasmodium gallinaceum* are injected intravenously into chicks, subinoculation of the injected chicks between a period of 15 minutes and about two days afterwards yields negative results. The development phase occurring during this period is the post-sporozoite phase or tissue phase. The parasites at this time have been called cryptozoites. *The tissue phase.* *Cryptozoites.*

It will thus be apparent that there are three possible targets in the chemotherapy of malaria namely, the sporozoite, the tissue phase, and the blood phase. Clinical symptoms do not appear until the blood phase, and as early attempts at chemotherapy were aimed at the alleviation of symptoms, they were naturally aimed at the blood phase. From this point of view quinine was the great specific in malaria, and it was a substitute for quinine that was being searched for in earlier experimental chemotherapy, and it was the efficacy of quinine in the experimental infections which formed the index for the utility of synthetic anti-malarial drugs. Thus it was the blood phase which was used for experimental work, and culminated in the development of the Roehl test for anti-malarial compounds. The search for drugs to kill either the sporozoites or the tissue phase, *i.e.,* a search for *causal prophylactic drugs,* drugs which would prevent parasites ever appearing in the blood, was neglected.

The Roehl test is based on the fact that when canaries are inoculated intramuscularly with *Plasmodium relictum,* a period of 4 to 8 days normally elapses before the parasites can be easily found in the blood of infected birds, but, if during the prepatent period, the infected birds are dosed with an active anti-malarial drug, the period is increased beyond the 8th day. In Roehl's actual experiments, dosing was started 4 hours after the inoculation of the parasites and continued once daily for the next five days. Delay in the appearance of parasites in the blood was regarded as significant if it extended to or beyond the 10th day. *The Roehl test.*

Certain malariologists were, however, not satisfied with this test, and were emphasizing that the urgent problem in chemotherapy was not to find a substitute for quinine, but the discovery of a drug which would prevent parasites developing as far as the blood phase, an accomplishment not possessed by quinine. Thus, while quinine and mepacrine were satisfactory supressive or clinical prophylactic drugs, what was needed now was a drug that would kill either the sporozoites or the phase intervening between the sporozoites and the blood phase, *i.e.,* the tissue phase. Further, it was shown that while certain of the sulphonamide drugs were active in human malaria, though their action was not as good as quinine or mepacrine, yet none of the sulphonamides could be shown to have any measurable effect when tested by the Roehl's method against *P. relictum.* A laboratory infection had, therefore, to be sought, which besides being sensitive to the sulphonamides, should also be sensitive to all the other types of drugs known to be efficacious in human malaria. Such infections were discovered in 1938 in the *P. gallinaceum* infection of chicks and *P. lophurae* in ducks. *Need for a new method of testing.*

P. gallinaceum infection in chicks possess the requisite qualities for experimental infection. This organism which was first described by Brumpt in 1935, who obtained his material from wild fowl of Indo-China. The parasite is easily maintained in a large, cheap, and *P. gallinaceum.*

easily kept laboratory animal. Its prominence was achieved through the work of James and Tate (1937) who described a stage in its life-cycle in the chicken, which existed in cells other than the red blood-cells. During this stage the parasites may occur in endothelial cells in all the tissues of the body, and are particularly plentiful in the brain, spleen, liver, lungs and kidneys. James and Tate suggested that such an exo-erythrocytic or tissue phase might exist in human malaria, and might explain the problem of relapses. The exo-erythrocytic forms have been described, definitely in bird malaria, but it is likely that in all forms of malaria the sporozoite after inoculation into the vertebrate host, develops first in the tissues, and only at the end of this tissue phase does the parasite invade the red blood-calls. This view may be represented diagramatically as follows:—

SPOROZOITES	(Free in blood-stream a very short time not more than about 30 minutes).
↓	
EXO-ERYTHROCYTIC FORMS	(Reproduce themselves by continual schizogony).
↓ ↑	
BLOOD FORMS	(Reproduce themselves by continual schizogony).

The P. gallinaceum test in chicks.

The P. gallinaceum test in chicks for assessing anti-malarial activity.—Six days old chicks weighing between 40 and 55 gm are inoculated intravenously with blood drawn from heavily parasitized chicks whose infection is not more than five days old. The inoculation should contain 40 to 50 million parasitized corpuscles, and results in an acute infection, the peak being reached on the fifth day when smears show that on the average 300 to 400 out of every 500 red-cells counted are parasitized. Using this method, the contrast between treated and untreated infections becomes very evident. A treated infection might rise slowly for a day or two, but then sinks to a low level, while the untreated one continues to rise rapidly and finally reaches the peak of the parasitaemia curve on the 5th day. At this stage the untreated infection may be 50 to 100 times as dense as the treated one. It is thus possible to detect even slight anti-malarial activity in a drug.

The time-table of the full *P. gallinaceum* test as described by Curd, Davey and Rose (1944) is as follows:—

Time-table of test.

"*Monday.*—(1) 9 a.m. Donor chicks with a four-day-old infection and showing at least half the red cells parasitized, are bled. The blood is then mixed with an equal amount of citrate heparin mixture and is further diluted with citrated saline to give a mixture containing approximately 45 million parasitized red cells in each 0.2 ccm.

(2) Selected chicks weighing between 40 and 55 gm are inoculated into right jugular vein with 0.2 ccm of the blood mixture.
(3) The birds are numbered and arranged in groups of six. Six birds are used for the trial of each dose of the drug, and for each control group.
(4) 4 P.M. (The first dose of drug calculated for a 50 gm chick and dissolved or dispersed in 1 ccm of water is administered by a catheter-tube passed into the gizzard.

Tuesday.—Dosing is repeated at 9 a.m. and 5 p.m.

Wednesday.—At 9 a.m. all birds are dosed; at 10 a.m. smears are made; at 5 p.m. dosing is repeated.

Thursday.—Procedure as on Wednesday.

Friday.—At 9 a.m. smears of control birds are made, stained and read. If the parasite-level indicates that the peak has been reached, smears are made of all birds. They are then stained with Giemsa, read, and the test ends."

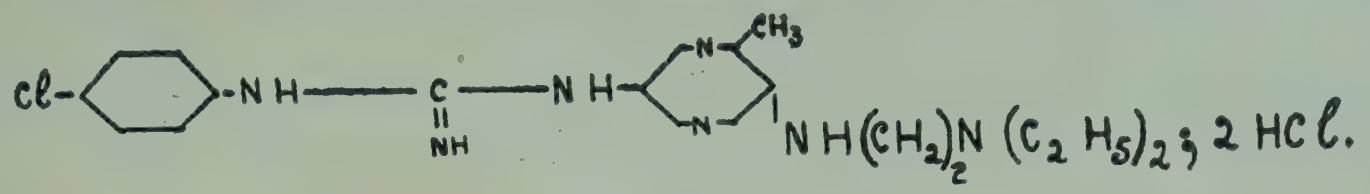
Use of mepacrine as a standard.

Mepacrine was selected as the standard anti-malarial drug against which the new drugs were to be compared for anti-malarial activity. The dosage of standard drug used was in the neighbourhood of the critical dose, which for mepacrine is in the region of 2 mgm/50 gm. The *critical dose* of anti-malarial drugs is the smallest dose which accomplishes practically everything the drug is capable of, and increasing this dose many times will not materially improve the results.

Activity of 2666.

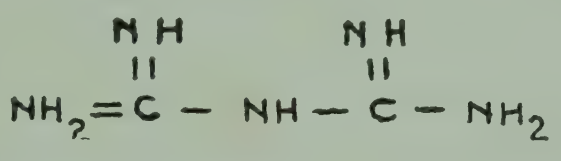
The first new anti-malarial drug, unrelated to any previously known drug, discovered was 2666.

Its critical dose region was found to be 2-4 mgm/50 gm, and its chronic toxicity for mice about two and a half to three times that of mepacrine. The drug was tried clinically in human malaria at the Liverpool School of Tropical Medicine, but even with the maximum tolerated doses, the results were negative. However, since 2666 was an entirely new type of anti-malarial drug, there was the possibility of increasing its anti-malarial power by further modifications of tthe nucleus. This was achieved in 3349, which differs from 2666 in having a guanidine linkage between the two ring systems, thus:— *Activity of 3349.*

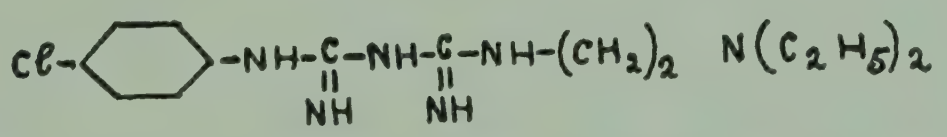


The critical dose region of 3349 and its chronic toxicity figures for mice were in the vicinity of the corresponding values for mepacrine, and its anti-malarial activity against *P. gallinaceum* was almost as good. On clinical trial against malignant tertian, benign tertian, and quartan malaria, in doses of 200 mgm thrice daily, the results were as good as could have been expected with quinine, and no toxic reactions were observed.

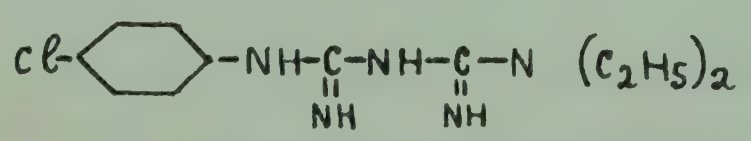
Following the synthesis of 2666 and 3349, Curd, Davey and Rose continued their studies on the relation of the chemical structure of these compounds to their anti-malarial activity. They also planned a stepwise simplification of these compounds in order to determine at which point anti-parasitic activity disappeared. Both these compounds were salts of strong bases and it was tentatively assumed that this property had to be retained in subsequent compounds. The 4-methyl group in the pyrimidine ring in 3349 was not originally thought to have any special significance, and it was proposed to omit it. If the function of the pyrimidine ring was solely to provide two tertiary nitrogen atoms suitably orientated to provide the necessary prototropism, then the entire—C(CH$_3$)=CH—unit of the pyrimidine ring might also be inessential, and it was suggested that the cyclic system could be dispensed with in this portion of the drug molecule. With these portions of the molecule omitted, 2666 then appeared as a derivative of biguanide *Synthesis of the bi-guanide compounds.*



and 3349 as a derivative of an extended biguanide. Accordingly a biguanide compound having the formula

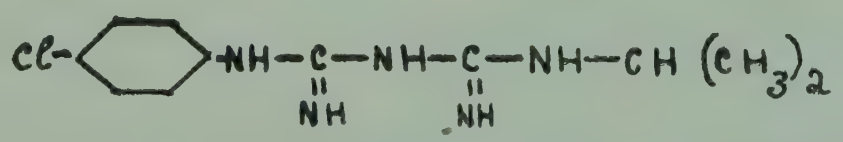


was prepared, but the product proved inactive in the chick test. However, it was thought that this might be due to the strongly basic properties of this substance. This was corrected and resulted in the compound 3936.



This substance showed marked anti-malarial activity.

Further researches on the biguanides resulted in the synthesis in November 1944 by Curd, Davey and Rose of 4888. This compound was found to possess the highest degree of anti-malarial activity of the whole series of these new compounds, and exerted a strong causal prophylactic action; 4888 has the formula N$_1$—p—Chlorophenyl—N$_5$—isopropyl



Biguanide hydrochloride and was named PALUDRINE. It is a colourless crystaline compound, freely soluble in water, and melting at 189°C.

(ii) Biological results

The following is a brief summary of the results obtained from all the work described above.

Extra-erythrocytic forms.

1. Sporozoites which are the means of transmitting malarial infection from the mosquito to the vertebrate host in all species of *Plasmodium*, have been shown in *P. gallinaceum* and *P. relictum* to undergo a cycle of development in the solid tissues and circumstantial evidence indicates that a similar cycle occurs in all species of malarial parasites. The forms which develop directly from the sporozoites are unpigmented and are called the primary *i.e.*, extra-erythrocytic forms or cryptozoites. Drugs are considered as having a causal prophylactic action if they are active either against the sporozoites or the primary *e.e.* forms.

No known drug has any action on the sporozoites of any species, but the sulphonamides are active against the primary *e.e.* forms of *P. gallinaceum*, although not against those of any other species, as far is as at present known.

4888 was also active against these forms of *P. gallinaceum*, but unlike the sulphonamides, was also active against the primary *e.e.* forms of *P. relictum*, *P. lophurae* and *P. cathemerium*. Its action was sufficiently general for the hope to be entertained that it would be causal prophylactic in human malaria. This hope was to a considerable extent justified by results of clinical trials now taking place in all parts of the world.

2. At the end of the period of development of the primary *e.e.* forms, there is a release of parasites into the blood-stream. In chicks this takes place about 36 hours after inoculation of sporozoites. In human malaria, the earliest times at which parasites have been recovered from the blood is about 6 days after inoculation of *P. falciparum* sporozoites and about 8 days after inoculation of sporozoites of *P. vivax*.

The density of the blood infection gradually increases, and at the same time the *e.e.* forms in the solid tissues also increase in numbers.

3. It is known that none of the standard anti-malarial drugs, such as mepacrine and quinine, have any apparent action on the cryptozoites (e.e. forms): and clearly, if these are present, they may serve as reservoir for the release of blood forms. It follows, therefore, that a causal prophylactic drug active against e.e. forms should also achieve radical cures. This point is in process of checking by using sulphadiazine or sulphamezathine and 4888 in a long series of experiments. All these substances possess marked casual prophylactic properties in infection of young chicks, and since none of them has any action on sporozoites, the prophylactic results are due to their action on e.e. forms. If treatment is begun early enough, all of them are capable of completely eradicating the infection, but 4888 is the only one with which treatment can be delayed until 2 or 3 days before death is expected, and the mortality-rate still be influenced. The difference between 4888 and the others in its effects on *P. gallinaceum* infections is probably one of dynamics of drug action, because all these substances influence the development of *e.e.* forms in infections of young chicks induced by the inoculation of infected blood. If such infections in 6-day-old chicks are treated for as long as possible with mepacrine and quinine the chicks commence to die after about 14 days, and death is due to blockage of the brain capilliaries with e.e. forms. These infections can, however, be treated with 4888 or sulphadiazine, and death of the chicks prevented. It seems that the differences between 4888 and the sulphonamides in their effects on infections with *P. gallinaceum* are due to the much more intense and much more rapid action of 4888 on the e.e. forms.

(iii) Extra-Erythrocytic Phase in Human Malaria

Studies on species of *Haemoproteus*, which is known to reproduce only in endothelial cells, showed that it was exceedingly difficult to demonstrate the presence of schizonts in the tissues unless these were examined soon after the infection with sporozoites had taken place. That such schizonts are present in the organs at other times is proved by Brumpt's observation of the reappearance of gametocytes in the blood after the blood has been cleared of these forms by the administration of plasmoquine. Further evidence of the development of exoerythrocytic stages from sporozoites is afforded by the lack of infectivity of the blood after injection of sporozoites. Blood taken from birds or human beings during the first four to six days after sporozoites infection fails to produce infection when injected even in relatively large amounts into uninfected birds or human beings. Such a negative phase does not occur when infections are produced by inoculation of infected blood. In this case the blood is immediately and continuously infective. This means that, as judged by blood infectiveness, there is a minimum incubation period of four days following injection of sporozoites, a minimum incubation period which cannot be reduced, however great the dose of sporozoites. When infected blood is inoculated, the incubation period is progressively reduced with every increase in dose.

As regards the merozoites resulting from the first and subsequent exoerythrocytic schizogonies, these vary in their disposition to enter other endothelial cells or the red blood corpuscles. In this respect they are "histotropic" or "hæmotropic." In *P. relictum* the first merozoites are highly histotropic so that the organs of birds examined soon after sporozoite infection reveal exoerythrocytic schizonts. The second and following merozoites are more hæmotropic and possibly not histotropic at all, with the results that exoerythrocytic schizonts become increasingly difficult to find. This is true of *Haemoproteus* and presumably of *P. faliciparum.* On the other hand in *P. gallinaceum* the successive batches of merozoites are highly histotoropic, so that exoerythrocytic schizonts are readily detected at all stages of infection. It would seem that *P. vivax* and *P. malariae* occupy an intermedite position in this respct.

Fairley N. H. (1947) working with the Medical Research Unit in Australia, carried out a large number of subinoculation experiments. Viable sporozoites were demonstrated in the circulating blood of volunteers for from half to one hour after inoculation of parasites by the bite of Anopheline-mosquitoes. This was followed by a period, lasting six days in P. falciparum and eight days in P. Vivax infections, during which pre-erythrocytic forms are undergoing schizogony in the reticulo-endothelial cells, and during which time massive subinoculations of blood from heavily infected individuals persistently failed to induce Malaria in recipients. It would thus appear highly probable that an exo-erythrocytic mechanism occurs in human malaria, though this has not yet been conclusively demonstrated. This would explain the temporary non-infectivity of the blood after injection of sporozoites, and would also explain why in naturally acquired malaria a short treatment of an acute attack with quinine or mepacrine although highly effective in clearing the peripheral blood of parasites, often fails to prevent a relapse. These drugs while highly potent against erythrocytic forms, are presumably inactive against the extra-erythrocytic forms and relapses may result from further invasion of the blood by the persisting unaffected exo-erythrocytic forms. This presumption is further borne out by the observations that artificially induced P. Vivax infections by inoculation of blood from infected patients, are readily cured by a short course of treatment, and relapses rarely follow, presumably due to absence of exo-erthrocytic forms.

Evidence adduced from chemotherapeutic experiments suggests that in human malaria, the exo-erythrocytic phase in P. falciparum infections, is a short one lasting only 6 or 7 days and Schizogony Cycles of this phase are few, perhaps only two or three, after which all the parasites convert into erythrocytic phase. Thus treatment of an acute attack of falciparum malaria by drugs acting on the asexual blood forms, if continued for three to four weeks, usually completely eradicates the infection. With P. vivax infections, however, evidence suggests that the schizogony cycles in the exo-erythrocytic forms may continue indefinitely and last for several months, and treatment however intensive of such infections with anti-malarial drugs, frequently fails to prevent relapses.

The above experiments showed that paludrine and plasmoquine are complete causal prophylactics in P. falciparum infections, but only partial causal prophylactics of P. vivax.

Raffaele, (1946) claims that he has demonstrated exoerythrocytic stages of development in *all* the three human parasites, chiefly in bone marrow taken by sternal puncture from patients a few days after infection by sporozoites. Only in the case of *P. malariae* has he been able to demonstrate such forms a year after infection had taken place. He notes that the existence of these stages accords an explanation of the failure of various drugs to prevent infection from sporozoites though the drugs are known to be active in the case of the erythrocytic stages of development. He shows that the demonstration of the exoerythrocytic forms in human malaria by himself and others establishes once for all the similarity of the human and avian parasites.

Angelini (1947) in a critical review of the various records of occurence in smears of the bone-marrow and other organs of exo-erythrocytic schizonts of human malarial parasites, is of the opinion that upto the present, no reliable morphological record of these stages in human malaria has been made. He finds that detached portions of cytoplasm of cells, as occurs commonly in smears of bone-marrow, may simulate these stages.

(iv) Pharmacology & Toxicity of Paludrine

Pharmacology of paludrine.

It has not been fully worked out but preliminary experiments have been carried out on its absorption and excretion in volunteers, who were given 50 or 500 mgm of the drug every 12 hours for 14 days. The drug is absorbed from the gastro-intestinal tract and maximum concentration in the blood is reached on the 4th or 5th day after its commencement. The concentration is then maintained more or less. The fall-away of concentration in plasma is very rapid after the drug is stopped but it can be detected in urine up to nine days after cessation. The urinary output roughly follows the same course and drug can be detected in the urine after 12 hours.

Toxic effects.

Paludrine has not been used in the treatment of malaria long enough to enable the clinician to find out its toxic effects. From the data collected so far it appears that its toxicity is very low. In such large dosage as 1.0 gm daily (three times larger than the effective therapeutic dose) paludrine when administered to volunteers with overt malaria may produce toxic effects which are not serious and which may be relieved by diminishing the daily dose or by cessation of therapy for 1 to 2 days. The chief toxic effects observed have been irritation of the gastro-intestinal and urinary tracts; vomiting occurs, and red blood cells, epithelial cells, and occasional hyaline or granular casts may be found in the urine.

Volunteers taking 500 mgm twice daily for 14 days who carried on their normal work, rarely complained of some abdominal discomfort and pain, appearing about 30 minutes after a dose, most noticeable in the first few days of the course. One volunteer vomited after the 5th or 6th dose. As much as 700 mgm twice daily can be tolerated provided the patients are kept in bed for first few doses.

A transient increase in myelocytes in the peripheral circulation which occurs on the 7th to 10th day after commencing therapy for overt malaria has been frequently observed. Whether this reaction can be interpreted as a direct toxic result of drug action or as specific stimulation by the drug of the myeloblastic tissue of the bone marrow recently released from the inhibitory effect of malarial infection is not clear.

Chen and Anderson (1947) made the following observations on the toxicity and general pharmacology of paludrine in various laboratory animals. After administration of the drug in various doses to rate, no pathological lesions could be demonstrated. In anaesthetized cats, the drug was found to lower the blood-pressure and accelerate the respiratory rate. The isolated rabbit's uterus and intestine and the isolated guinea pig's intestine are relaxed, but the isolated guinea pig's uterus responds by contraction. The action on the blood-sugar of rabbit is to produce a slight hypoglycaemia.

(v) Paludrine in human malaria

(1) A large series of experiments have been performed to determine the anti-malarial activity of paludrine both in volunteers infected with strains of *P. vivax* and *P. falciparum* and natural infections. The effective therapeutic range of the drug is wide and in doses ranging from 10 to 700 mgm twice

daily, it cured the clinical attacks quickly and effectively. The mean time taken for clinical recovery as determined by feeling of well being and fall of temperature to normal and for disappearance of plasmodia from the blood was approximate to that with quinine and mepacrine. In dosage of less then 10 mgm twice daily it was not uniformly effective in dealing with the clinical attack. This extremely wide therapeutically effective range of dosage is in marked contrast to the limited rage of quinine and mepacrine.

2) Paludrine has proved superior to all known anti-malarial drugs as in non-toxic dosage, it is a true causal prophylactic, exerting a powerful lethal effect on extra-erythrocytic forms of *P. falciparum* and fully protecting volunteers receiving viable sporozoites by mosquito-inoculation. In *P. vivax* sporozoite-induced infection, it is only a partial causal prophylactic, the extra-erythrocytic forms are inhibited but eradication does not regularly occur. The only other drug which has a similar action is plasmoquine, but this drug has to be given in a dosage which is too dangerous for routine use in man.

3) Paludrine in non-toxic dosage controls malarial fever and terminates the clinical attack. It is a powerful schizonticide both in vivax and falciparum malaria. It appears to act on the early schizonts interfering with nuclear (chromatin) division. It produces radical cure in malignant tertain malaria, 64 out of 65 individuals with sporozoite-induced infections being completely cured even by 0.1 gm daily for 14 days. The Cairns results suggest, however, that where individuals are not cured, the weekly administration of 1-2 tablets (0.1-0.2 gm) indefinitely would prevent relapses until cure was attained.

4) Though not gametocidal in the accepted meaning of the term, sterilisation of the infection occurs in mosquitoes fed on M.T. or B.T. gametocyte carriers a few hours after taking the drug. This sterilization effect persists while the drug is being taken and for variable period after administration of the drug ceases, depending on the dosage adopted. Its action in this respect is superior to quinine or atebrin, for gametocyte carriers will continue to infect mosquitoes while these drugs are being administered. On the other hand as a true gametocide, it is inferior to plasmoquine.

5) As a result of extensive field trials by Fairley (1946, 1947) working with the Medical Research Unit of the Australian Army, it was established that for clinical cure of all types of malaria, 300 mgm given in a single dose generally suffices. In faliciparum infections 100 mgm three times a day if continued for ten days completely eradicates the infection in practically all cases. For vivax infections, owing to the long persisting exo-erythrocytic phase, no short course of the drug will certainly guarantee against relapse, and it is advised that 100 mgm three times a day be given till the acute attack has been controlled, and thereafter a single dose of 100 mgm weekly be given for a further period of six months, by which time it is hoped the infection will be exhausted. In all *P. vivax* infections and in the vast majority of *P. falciparum* infections, there would be too few falciparum gametocytes readily to infect mosquitoes after this 33 days period had elapsed.

6) *Causal prophylaxis and suppression.* Paludrine in dosage of 100 mgm weekly proved adequate for the full suppression of malaria in volunteers repeatedly exposed to heavy infection by mosquitoes with viable sporozoites, of *P. vivax* and *P. falciparum* in their salivary glands. Subinoculation arising from the extra-erythrocytic forms is inhibited in both vivax and falciparum infections, this being fundamentally different to the suppressive effects of atebrin,

sanctochin, resochin, quinine, and sulphadiazine. With the latter group of drugs, suppression or cure is achieved by schizonticidal action, where as with paludrine there is a direct effect on the extra erythrocytic forms as well. In falciparum malaria the extra-erythrocytic forms are destroyed and radical cure results (complete causal prophylaxis). In vivax malaria with a regimen of 100 mgm weekly, the delaterious inhibitory effect is temporary, the extra-erythrocytic forms surviving and later giving rise to erythrocytic parasites and overt malaia (partial causal prophylaxis).

7) The difference between the effective therapeutic dose and the toxic dose is very considerable. Minor toxic effects have been noted to follow the administration of 300 mg daily for the therapy of overt malaria. These consisted of vomiting and signs of irritation of the renal tract. These toxic phenomena rapidly disappeared on reduction of dosage or subsidence of the overt attack of malaria. A transient increase in myelocytes with maximal rise on the 7th-9th day was frequently observed. In lower dosage regimens, such as 0.3 gm daily for 14 days, significant toxic symptoms were absent.

Result of trials in India.

Paludrine has been tried in India under the direction of malaria Institute of India. According to Covell it is the only drug that can be used with entire safety to ensure complete causal prophylaxis against malignant tertian malaria, having a definite lethal action on the pre-erythrocytic forms. In benign tertian infections it acts as a partial causal prophylactic only. It will however effectively suppress all forms of malaria when given as a single dose once, or at most twice, weekly. It is a powerful schizonticide in benign and malignant tertian malaria and a single dose of the drug will control the clinical attack of both these infections, though there is as yet some doubt as to its efficacy in quartan malaria.

"Paludrine exerts no obvious primary effect on either the number or microscopical appearance of gametocytes in the patients' blood, but mosquitoes feeding on individuals taking the drug are unable to transmit the infection.

'Paludrine is remarkably free from toxic complications, its range of activity measured as the ratio between the effective therapeutic and the toxic dose being far greater than with any other antimalarial drug yet known. This property, together with its action in controlling the clinical attack with a single dose and effecting complete causal prophylaxis in malignant tertian infections render it particularly suitable for widespread use under both rural and urban conditions throughout India.

"As regards the prevention of relapse in benign tertian and quartan malaria paludrine possess little, if any, advantage over other antimalarials. Since however the occurrence of a relapse can be prevented by administering a small dose of this apparently harmless drug once a week, there seems no reason why this should not be continued until the infection has completely died out."

A number of other new anti-malarial drugs have also been prepaired and tested.

(2) Chloroquine (S. N. 7618)

Chloroquine.

Chloroquine was first synthesized in Germany some years ago, but was considered too toxic for general use and was slightly modified under the name of Sontochin. During the recent war supplies of the latter drug were captured in North Africa by the Allies and were subjected to detailed investigation in U.S.A. The Americans found Sontochin effective in human malaria but did not agree with the original German verdict on Chloroquine, and thought the latter superior and worthy of further development.

"Chloroquine is a member of the 4-aminoquinoline series and has the formula 7-chloro-4-(4-diethylamino-1-methyl-butyl-amino) quinoline. Large scale trials have proved that it will terminate a clinical attack of benign tertian malaria more abruptly and cure malignant tertian malaria more promptly than mepacrine. It appears to act in the same way as mepacrine but to be more effective, and is said to be less apt to cause disagreeable gastro-intestinal symptoms. It is an effective suppressive drug, when administered in a single dose once weekly.

The Medical Department of the United States Army has now adopted Chloroquine-diphosphate as a standardized "improved malarial therapeutic and suppressive drug." It is supplied in tablets of 0.5 gm., each containing the equivalent of 0.3 gm. of the base. The standard dosage adopted is as follows: For an initial attack or acute relapse one tablet of 0.5 gm is given initially, followed 4 hours later by another 0.5 gm. For the next three days one tablet of 0.5 gm is given each morning at 9 A.M. (total dose 2.5 gm in 4 days). It is recommended that this dosage should not be exceeded. For suppressive chemoprophylaxis one tablet 0.5 gm is recommended weekly. With this regimen the blood is cleared of parasites in less than 24 hours and the interval between relapses is stated to be considerably longer than with quinine or mepacrine therapy. Toxic symptoms are stated to be few, infrequent and mild, and rarely necessitate discontinuance of therapy. Anorexia, mild nausea and vertigo were rarely encountered, and the only common toxic manifestation was pruritis without rash in less than ten per cent of the cases.

(3) Metachloridine (S. N. 11,437)

Metachloridine.

"The formula for this drug is 2-metanilamido-5-chloro-pyrimidine, which is somewhat similar to that of sulphadiazine. It is very active against several species of avian malaria. In human malaria it has been disappointing in the treatment of malignant tertian infections but is said to be active in quartan. As mentioned above, there is some evidence to suggest that paludrine is less effective in quartan infections than in benign and malignant tertian, and should this be confirmed metachloridine may prove of value in the treatment of quartan malaria, either alone or in combination with other antimalarial drugs. Unpublished reports on limited series of cases indicate that it has a very low grade of toxicity, but further evidence is required before a definite opinion can be given on this point!"

(4) Pentaquine

Pentaquine or 'SN 13,276' is a quinoline derivative having the chemical formula 6-methoxy-8-(5 isopropylamino-amylamino)-quinoline, and is used as the diphosphate salt. The drug is rapidly absorbed from the gastro-intestinal tract of experimental animals and appears to closely resemble plasmoquin in its absorption, excretion, and tissue distribution. In man also, it is rapidly absorbed, plasma levels are quickly attained and fall to zero within 24 hours of stopping the drug. Pentaquin is only one third as toxic as plasmoquin in the rat, half as toxic in dogs, and half to quarter as toxic in monkeys. The toxic reactions are similar to those produced by plasmoquin.

Antimalarial activity.

Pentaquin was found to be more active than either quinine or plasmoquin against *P. gallinaceum* and *P. lophurae* infections. In experimental trials in man with P. vivax infections, the drug was found to be at least partially effective as a causal prophylactic when administered in toxic doses (120 to 180 mgm a day) to persons bitten by heavily infected mosquitoes on the second day of drug administration.

As a curative, pentaquine was found to terminate individual attacks, but was only partially effective in preventing relapses when given alone in doses as high as 120 mgm a day. When combined with 30 grs (2 gm) of quinine a day, however, doses of 60 mgm a day of the drug were found to achieve complete eradication of the disease in 16 out of 17 patients so treated.

Dosage.

Pentaquine is too toxic a drug to justify its use for either prophylactic purposes or for prolonged suppressive therapy. For cure of *P.vivax* infections, however, a daily dose of 60 mgm of the base combined with 30 grs quinine daily, in divided doses every 4 hours for 14 days, is sufficient to produce radical cure of severe vivax infections. The daily dose of 60 mgm of the base (80 mgms of the diphosphate salt), should not be exceeded, and the drug should only be given under close medical supervision. Besides plasmoquinine and paludrine this is the only other drug which has been shown to have action on the exoerythrocytic cycle.

(5) "Cam-Aqi"

"Cam-aqi," 4 (3 diethylamino-ethyl 4-hydroxylanilino)-7 chloroquinoline dihydrochloride dihydrate, is yellow crystalline powder introduced by the Parke Davis Research Laboratories, and found by them to be "several times as effective as quinine and quinacrine in avian malaria." Absorption from the gut was very rapid and high plasma levels were rapidly reached. The acute toxicity in laboratory-animals was about half that of mepacrine, and the chronic toxicity even less.

Simmeons and Chhatra (1947) tested the drug in human malaria in dosages of 10 mgm per kilo body weight. In their very small series of cases they found that the blood was cleared of malarial parasites in 24 to 48 hours. Further reports will enable the value of the drug to be assessed.

13. Summary of treatment of Clinical Malaria

General management:—The patient should be put to bed when the paroxysm starts, preferably in a dark well ventilated room. Bedding and clothing should be such as to give the least discomfort when the patient is drenched with sweat. If possible the clothing should be changed frequently.

The diet should be light with plenty of carbohydrate; glucose may be given. During febrile period only fluid should be given and plenty of water to drink in form of lemonade. When fever subsides full diet should be given. The bowels should be kept working daily with calomel and salts; in all cases a preliminary purgative should be given. Aspirin may be given to relieve headache. Proper nursing is important, and should be started immediately without waiting for result of blood examination.

Quinine:—Ten grains twice daily (20 grains in 24 hours) are quite sufficient in an adult Indian patient, but in Europeans ten grains three times a day (30 grains in 24 hours) may have to be given daily. In children and women, smaller doses are required. The drug should be combined with alkalis and continued for 7 to 10 days. Quinine mixture—Quinine sulphate or totaquina, 10 grains, citric acid 30 grains, magnesium sulphate 60 grains, distilled water one ounce. Alkaline mixture—soda bicarb 60 grains, sodium citrate 40 grains, calcium carbonate 3 grains and distilled water one ounce. The alkaline mixture is given half an hour before the quinine mixture.

In malignant forms, quinine should be given immediately by the intravenous route in doses of 7½ to 10 grains; 1 to 3 injections may have to be given to produce disappearance of parasites from the peripheral blood. After this the quinine should be given by the mouth and if this is not possible, by the intramuscular route till oral administration can be given. The treatment should be continued for 10 days. For intramuscular use quinine may be combined with urethane to decrease the pain due to the acidity of the solution—Quinine hydrochloride 10 grains, urethane 5 grains and sterile distilled water 2 ccm.

Atebrin—This drug should be given in doses of 0.1 gm (1½ grains) three times a day for 5 days (according to some for 7 to 10 days) in an adult. If crescents are present the course should be followed by 0.01 gm of plasmochin twice daily for 3 to 4 days. In malignant cases 0.1 to 0.3 gm of atebrin in soluble form should be given intravenously or intramuscularly followed by oral administration. If relapses occur the whole course laid down above should be repeated with each relapse.

Paludrine:—Give 100 mgm of paludrine thrice daily for 10 days in case of M.T. infection; this will produce a radical cure. In case of B.T. infection similar doses have to be given but 100 mgm once a week may have to be given for some time afterwards to produce a radical cure.

The following is the schedule of dosage recommended by the manufacturers for Indian strains of malaria: for clinical cure of all types of malaria, 300 mgm (3 tablets) in a single dose; for radical cure of malignant tertian infections 100 mgm three times a day for ten days; for radical cure of benign tertian infections, after the acute attack has been controlled, 100 mgm are recommended to be given twice weekly for six months. For children over 10 years of age the same dosage as for adults is recommended.

Chloroquine diphosphate—For an initial attack or acute relapse, on the first day two doses of 0.5 gm are given with a 4—hours interval. On the next three days a single dose of 0.5 gm is given each morning (total dose 2.5 gm in 4 days). This dosage should not be exceeded. This drug is being extensively used by the U.S. Army, and with the above dosage the blood is generally cleared of parasites in less than 24 hours.

14. Prophylaxis of Malaria

(1) General Measures

Anti-Mosquito Measures.—It is more economical to concentrate on the elimination of the main human malaria carriers rather than to campaign against all mosquitoes. Such small doses of plasmoquine as 0.01 gm twice daily for a few days sterilize the sexual forms of *P. falciparum* so that they can no longer infect mosquitoes. Sexual forms of other plasmodia are damaged by quinine and atebrin.

Elimination of mosquitoes.

Some species of mosquitoes enter houses and prefer to feed on domestic animals (keeping of cattle near houses may afford protection to man). The breeding of dangerous species is checked by measures based on the study of habits of each species. In some species, such as *A. minimus*, shading of streams prevents breeding. Rice fields, where the water is kept in continuous motion, are particularly dangerous and in Java the drying-off of the fields throughout a

tract one day each week has been made obligatory. This is effective without damaging the crop.

Bright and well ventilated houses, with no dark corners, discourage the entry of mosquitoes; rooms may be made mosquitoe proof if possible with wire gauze of 18 mesh. Swatting of resting mosquitoes and spraying the walls with insecticides are useful measures. Recently the "Freon bomb", a small round drum containing pyrethrum concentrate dissolved in compressed freon gas, has been evolved by the American Army. On opening the nozzle, the insecticide is projected 6 to 8 feet in the form of a fine fog or aerosol, which kills all mosquitoes in 100,000 cubic feet. D.D.T. is another recent and very efficient insecticide.

Larval control.

Larval control is carried out by getting rid of all collections of stagnant water in or near the houses which form the breeding ground. A preliminary survey by an expert is essential if large scale operations of mosquitoe destruction are to be carried out, as even such measures as clearing of jungle or drainage may produce increase of the mosquitoe vectors. Drainage of marshes and collections of water, filling of burrow pits, training of streams, alternate flooding and flushing of streams, etc., protection of wells and cisterns, snch biological measures as introduction of fishes which feed on mosquitoe larvae, oiling and use of chemicals are some of the methods employed. The operations should extend well beyond the area to be protected.

A wide variety of oils are used for destruction of mosquito larvae. Kerosine oil is largely employed either alone or with the addition of 1 to 2 per cent cocoanut or castor oils to improve its spreading power. Anti-malarial oil (A.M.M.) is a mixture of diesel oil, solar oil, and kerosine.

Malariol, a mixture of petroleum, and *waste motor oil* has also been extensively used. Oiling kills mosquito larvae by suffocation and by its toxic action. The oil may be applied by spraying under pressure through an atomizing nozzle. Oil swabs or cotton wool steeped in oil and weighted down by a stone when thrown into water are ideal for springs and running streams. A "gudda" made of sacking will soak up 2 gallons of oil and last 3 months, the oil gradually exuding and spreading as a fine film over the surface of the water.

Paris green (copper aceto-arsenite) is mixed with one hundred times its weight of finely sifted road dust, soapstone or powdered charcoal, and is sown by hand, down wind over the water surface at the rate of 17 grains of Paris green per square foot of surface. The particles are eaten by the anopheline larvae and act as a direct poison. In the quantities used, this preparation is harmless to other forms of aquatic life. In America, special dusting machines and aeroplanes are largely used for this purpose and an aeroplane equipped with a special automatic machine, can cover 20 square miles a day. *Greenglide* another arsenic-copper mixture (arsenious oxide 55.37 per cent, copper oxide 31.12 per cent, water soluble arsenic 1 per cent) marketed by Graven and Co. has an advantage over Paris green in that it will float for weeks. *Copper sulphate* is especially useful for tanks where drinking water is stored, or where sheets of algae are sheltering the larvae.

Mosquito-netting.

Mosquito-netting, containing '25 to 26 holes to the square inch' should be employed to cover beds at night and edges of the net either tucked in or so weighted that they touch the floor. During the day time, the net should be rolled up so that no mosquitoes are entrapped.

Mosquito repellents.

Pyrethrum powder is most effective for fumigation and may be used dry and puffed into the air of a room with a handspray or a nebulizer. Many proprietary sprays, are on the market and most of them are mixtures of kerosine oil and pyrethrum. The following may be mentioned: Flytox, Shell-tox, Pyrotox, etc.

Mosquito repellents.—Many oils and ointments, which are smeared on the hands and race, are advocated for personal use. These usually contain either oil of citronella or pyrethrum extracts, or a combination of both. A suitable formula is Stearin Cream, which is non-greasy and gives 97 per cent protection for 8 hours. It contains stearin 16 per cent, 2 per cent pyrethrum extract, 10 per cent, glycerine 4 per cent, 33 per cent ammonia in water 0.9 per cent, and water 69 per cent. A similar preparation used in India contains citronella oil ½ oz, spirit of camphor ¼ oz and petroleum jelly ½ oz. An emulsion of kerosine and ordinary soap affords some protection when nothing else is available. The following cream is effective and keeps the mosquitoes away for at least 5 to 6 hours:—Stearic acid 27 parts, caustic

soda 0.77 part, gum tragacanth 4.8 part, pyrethrum extract one per cent 16 parts, oil of citronella 6.16 part, aqua 100 parts.

The education of the public especially students in the schools by simple illustrative posters, magic lantern lectures, cinema films, etc., is most important. The people in endemic localities should be made malaria minded.

Of the newer mosquito repellents recently introduced, *dimethyl phthalate* and *dibutyl phthalate* have been found best in practice, and when thinly applied to the skin, they have been found to give full protection for six hours or more. Both of these chemicals can also be used after incorporation into creams, but there is little advantage in this. They cause smarting if allowed to get into the eyes or into abraded skin. It must be remembered that these agents are powerful solvents of plastics and care should be taken to avoid contact with such plastic articles as spectacle-frames, fountain-pens, watch-glasses or artificial silk stockings. Another of the newed repellents is *Indalone,* which is used as a 25 per cent solution in Arachis Oil.

D.D.T. or Dichlor-diphenyl-trichlorethane is widely used for the destruction of larvae and adult mosquitoes, and exerts its lethal effect probably through its action on the peripheral and muscular systems. The action is slow, but persists for long periods. After a single spraying in the concentration of 100 mgm per square foot, the mosquito popullation of treated hours is greatly reduced for two to three months as compared with one week with the use of Pyrethrine alone. D.D.T. may also be incorporated with the whitewashing or paint applied to the walls.

In 1942, *Gammexane,* a gamma isomer of hexachlorocyclohexane, was introduced as an insecticide of high potency and low toxicity to animals and man. It is an excellent larvicide, and in the form of a spray-solution in paraffin, is very effective against adult mosquitoes. Extensive field trials in Sierra Leone by Davidson (1947) demonstrated its efficacy in malaria control by adult mosquito destruction. A 0.5 per cent solution applied at the rate of 10 mgm per square foot to the internal wall surfaces of houses, reduced the mosquito population of such houses to almost nil for a period of six months.

(2) Chemo-therapeutic Suppression and Prophylaxis of Malaria

Cinchona alkaloids.

Quinine (cinchona febrifuge and totaquinine) are used in India and other malarial countries as a prophylactic, and though these have considerable value, the protection afforded cannot be considered absolute. Under war conditions quinine prophylaxis has not proved satisfactory for troops operating in open warfare, and it should not be solely relied upon.

Six grains of quinine hydrochloride or the sulphate, should be taken about 2 hours before bedtime daily, during the malarial season. Koch's system advises 15 grs twice weekly on two consecutive days during the week. These doses, are, however, now considered insufficient and 10 grs daily are advised on entering the hyperendemic area, and continued for one month after leaving. Taken in this fashion the clinical symptoms of subtertian malaria are mitigated and the risk of blackwater fever is reduced.

The following figures cited by Celli, indicate the relative value of prophylactic measures:—Mosquito protection plus quinine prophylaxis, 1.76 per cent infected; mosquito protection alone, 2.5 per cent infected; quinine prophylaxis alone, 2.5 per cent infected; no protection at all, 33 per cent infected.

Experimental scientific evidence of prophylactic value of quinine is not very satisfactory. It has been shown that the sporozoites injected by an infected mosquito are not harmed even when patients are taking 30 gr of quinine daily. Nor has it any action on the extra-erythrocytic cycle of the parasite. It is not, therefore, a true prophylactic its action being suppressive on account of its destructive action on the cycle of the parasite in the blood.

Plasmoquine.

Plasmoquine attacks the gametocyte stage (even in doses of 0.01 gm twice daily for 2 or 3 days') and it was hoped that it would prove an ideal prophylactic. This drug also has no action on the extra-erythrocytic cycle and has no causal

prophylactic value. Even for suppressive prophylaxis such large doses have to be given which produce toxic effects.

Atebrin.

Mepacrine hydrochloride given in doses of 0.3 gm daily over a period of 5 to 7 days before being bitten by infected mosquitoes, considerably prolongs the incubation period of an attack. For complete effectiveness, the dosage has to be increased to a total of 6-8 gm over a period of 22 days before and 30 to 60 days after exposure.

For clinical or suppressive chemoprophylaxis 0.4 to 0.7 gm of atebrin weekly (0.1 gm daily) or 0.2 gm twice a week is said to be sufficient and does not interfere with general health. For children, doses ranging from half a tablet (0.05 gm) weekly for infants aged 2 years, up to two tablets weekly for children of 8 years of age, are advised. The effect of atebrin prophylaxis may be judged by the continued absence of clinical symptoms of malaria, the continued absence of malarial parasites in the peripheral blood, and the stability of the haemoglobin index, inspite of exposure to malarial infection. To be effective the atebrin level in the blood should be between 25 and 30 micrograms per litre of plasma. This drug, like quinine, has a suppressive action. In suppressive doses, atebrin is harmless, though some authorities assert that it may give rise to a debilitating diarrhoea or abdominal pain. Atebrin in suppressive doses given for up to one year, does not cause liver or renal damage.

The following conclusions were drawn from experimental investigations carried out in Australia during the War:—

Quinine sulphate given in solution, 10 grains daily, commencing two days before the first infective bite, failed to prevent overt attacks of *falciparum* malaria. Similar doses completely suppressed *vivax* attacks in some cases but not in all.

Certain sulphonamides (sulphadiazine, sulphamerazine and sulphamezathine) in doses of 1.0 gm daily, suppressed *falciparum* malaria in 20 out of 21 volunteers, and cured 17 of the 21. These drugs are not causal prophylactics; they destroy the asexual blood parasites. They failed to suppress *vivax* infections.

Mepacrine, 0.1 gm daily, suppresses *falciparum* malaria, and if continued for the requisite period after the last exposure to infection, cures the disease. Mepacrine in these doses also suppresses *vivax* malaria, but clinical attacks regularly occur a few weeks, and exposure of the subjects to ardous conditions comparable to those experienced on active service, did not rob mepacrine of its efficacy.

A correct mepacrine regimen may secure the control of malaria in hyperendemic areas. This knowledge has been applied with dramatic results. In December 1943, the malaria rate in Australian troops in New Guinea was 740 per 1,000 per annum; in November 1944, this rate had fallen to 26. Other causes had been at work; there was less fighting in 1944, and antimosquito measures were more effective but the absence of frequent *vivax* relapses indicated a high standard of mepacrine discipline.

The following procedure of chemoprophylaxis was adopted in the Royal Navy:—

Suppressive treatment with atebrin (mepacrine) is recommended for naval personnel who are exposed to malarial infection. One tablet of 0.1 gm (1½ grains) should be taken daily at the evening meal. This routine should be continued throughout the period of exposure to infection, and for a month afterwards; if, however, there is a high incidence among the men, this suppressive treatment should be continued while they are in the theatre of war whether based on a malarious or a non-malarious area.

In some persons mepacrine causes nausea and vomiting or colic and diarrhœa, but this is an initial intolerance, often due to taking the drug on an empty stomach or in too large a dose; temporary reduction of the dose leads to tolerance, and less than one per cent of persons are unable to continue taking it.

The routine should be modified in certain circumstances: when clinical attacks occur they are treated and the suppressive doses are resumed afterwards; if the incidence of

clinical attacks is high, say after return from strenuous combat, it may be reduced by increasing the daily suppressive dose to three tablets (0.2 gm) daily after meals for 3-5 days, and then returning to 0.1 gm a day as before.

Paludrine Hydrochloride has been found to be an effective causal prophylactic and chemo-therapeutic suppressive as a result of extensive clinical trial. The manufacturers recommend a dosage of 100 mgm (1 tablet) twice a week in hyperendemic areas, and 100 mgm once a week in others. For children below 10 years of age, half the adult-dose is satisfactory. With such suppressive doses even when continued for prolonged periods, toxic symptoms are not encountered.

Chloroquine diphosphate. For suppressive chemo-prophylaxes as a result of extensive clinical and field trials, the U. S. Army Medical Department recommend chloroquine diphosphate in a dosage of 0.5 gm weekly.

(3) Prevention and Control of Malaria in World War II

According to Saperol (1946) the experience and research during World War II have made important contributions in connexion with prophylaxis and control of Malaria. There is more effective anopheline control by the advent of D.D.T. There is more effective drug prophylaxis as a result of the development of new drugs and improved methods in the use of drugs which formerly produced only mediocre results. It should, however, be remembered that effective malaria control is attained only to the extent to which organized methods are employed and carried out by a team of specialists.

Role of DDT.

Prior to World War II, the main attack on anophelines was almost solely confined to measures which would destroy larval forms. Measures against adult mosquitoes were difficult and infrequently attempted. With the introduction of DDT, however, the attack has become twofold, *i.e.*, against both larvae and adults.

In the attack against the larval stages, the advantage of DDT, has been found to rest principally in the efficaciousness of light applications. For example; 2 quarts per acre of a 5 per cent solution of DDT in diesel oil will now suffice to accomplish as effective a kill as that previously obtained by 20 to 40 gallons of oil without DDT. These light applications thus enable an important saving of oil, of time and of labour to be effected.

Where there are large areas to cover, airplanes have proved effective for the dispersal of DDT. These wartime trials indicate that airplane in the postwar period will serve as an important aid to malaria control, not only as a device for saving time and labour but also for the control of areas otherwise inaccessible.

In the use of DDT. to control adult mosquitoes, many promising possibilities are being explored. During the war military forces found that by impregnating bed nets, by spraying the inside of tents and other types of housing and, particularly, by spraying native houses a significant reduction of the adult mosquito population could be accomplished. The attack on adult mosquitoes means that malaria transmission in an uncontrolled area can be stopped far more readily than has ever before been possible. Current experiments in which complete control is being attempted solely by adult kill show that whenever larvicidal measures are impractical for reasons of cost or inaccessibility, effective control by adult eradication can nevertheless be achieved.

This kill of adults is accomplished by the prolonged residual action of DDT. Another method of kill developed during the war is the use of aerosols containing DDT. Under favourable conditions, these aerosols are capable of killing both adult mosquitoes and larvae in a single operation.

Repellents

A great deal of wartime research was directed toward the development of new repellents. Unfortunately, repellents at present appear to have so many limitations that their general usefulness for protection against malaria does not seem promishing. It is difficult to be certain that persons will use repellents regularly enough to afford any real degree of protection. Any measure of malaria control which relies on individual initiative is bound to be ineffective. Another inherent limitation to the use of repellents is that those which are effective against one species of mosquito frequently are largely without effect against another. Present day repellents, however, are vastly superior to those used in the past and will be beneficial if used carefully.

Drug Prophylaxis.

Wartime experience and research has greatly extended the usefulness of drugs for prophylactic purposes. It is now possible for military forces to operate in highly malarious, uncontrolled areas without significant malarial morbidity.

It has been demonstrated that (*a*) both mepacrine hydrochloride and quinine are fully preventive against malaria due to P. falciparum merely by prolonging the administration of the drug for approximately a month after the last exposure; (*b*) in malaria due to P. vivax these drugs are capable merely of suppressing clinical symptoms, and that overt attacks are inevitable when administration of the drug ceases; (*c*) mepacrine hydrochloride is superior to quinine as a suppressive, and (*d*) the mechanism of protection is not due to a destruction of infective organisms but to the reduction of parasitaemia to a level insufficient for the production of clinical symptoms.

It has further been shown that various factors, such as combat fatigue or any other type of hard physical exertion, extreme cold blood loss and other factors do not precipitate "clinical break-throughs" in persons taking a drug as a suppressive measure. Failures with quinacrine hydrochloride which is taken in the recommended suppressive dosage are extraordinarily rare. Clinical break-throughs are due to failure to take the drug, in the vast majority of cases.

This knowledge gained during World War II has important implications in the present day control of malaria in civil communities *e.g.* labour forces working in highly endemic areas. Where malaria due to P. falciparum is prevalent, complete protection will be afforded. With infections due to P. vivax, the eventual problem of curing infections which have been temporarily suppressed must be faced when suppression by means of the drug is discontinued.

New Drugs.

Mepacrine hydrochloride has the serious drawback of staining the skin. In addition, many persons, on initial administration of the drug, experience gastrointestinal symptoms. For this reason, plus its inability to render any more than temporary protection in malaria due to P. vivax, attempts have been made to find other drugs which would not have these shortcomings. In this respect, chloroquine, has the advantage that it will not stain the skin, nor does it so commonly lead to gastrointestinal symptoms as does mepacrine usage. Further it needs to be administered only in a single weekly dose. This is exceedingly important, as careful supervision of weekly administration is much more feasible than the daily supervision required in the use of mepacrine. Chloroquine, like mepacrine hydrochloride, permits suppressive cure in infections due to P. falciparum and affords only temporary protection against malaria due to P. vivax.

The two drugs, however, are not causal prophylactics. Paludrine in P. falciparum, acts as a true causal prophylactic. Preliminary experiments against the

infections due to P. vivax indicate that it exerts at least a partial causal prophylactic effect and does so in doses which appear to be nontoxic.

Entomologic Control.

A team of expert workers is required for the control to be effective and economical. The entomologist is able to locate the single species transmitting malaria and to concentrate efforts on the species. In addition to the entomologist, a malariologist directed the overall program and an engineer carried out control operations.

The present day significance of these wartime achievements in the control of malaria is that they re-emphasize the fact that a like approach in civilian communities is essential. With such methods, according to Russell "one may reasonably hope that, with suitable organization, malaria will be eradicated from the United States within next decade, and that in many tropical areas, even though economically depressed, this disease, now of the greatest importance, may become in the next half century one of the least of public health problems".

15. Special Forms of Malaria

Pernicious Malaria.

The pernicious types are the gravest forms of malaria. In a patient with a single simple infection, pernicious and mild attacks may alternate. Almost all cases of pernicious malaria are produced by the malignant tertian parasite; only in rare and exceptional cases the benign tertian and quartan parasite may produce pernicious symptoms and death may result. The cases of pernicious malaria occur usually in highly malarious places and in the most malarious season of the year, but they may occur in places where the disease is not so intense. The pernicious symptoms come on sometimes with dramatic suddenness.

Symptoms produced are due to the internal sporulation of the parasites and the clumping of the infected red cells causing plugging of the capillaries in the brain, lung, liver, heart, intestine, spleen and the bone marrow. Thus a variety of pernicious manifestations which may simulate almost any disease, follow. The following however, are the well recognised types of pernicious malaria:—

Cerebral Malaria.

I. Pernicious malaria is called cerebral when symptoms of cerebral involvement are dominant. It is due to aggregation of red cells infected with malarial parasites in the brain which lead to inflammatory and degenerative changes. The peripheral blood may not show a heavy infection; the occurance of more mature forms, especially the schizonts of *P. falciparum* in the blood, however, is of grave significance and is an indication of threatening cerebral malaria. The symptoms set in during the course of fever, rarely in the beginning, and there is a history of mild intermittent fever. Such symptoms as nervous twitchings, light-headedness and drowsiness usually precede the onset of graver symptoms of cerebral types. The following are the sub-types of cerebral malaria:—

Hyperpyrexial.

(*a*) In hyperpyrexial type, the temperature instead of stopping at 104° or 105°, may continue to rise and passing 107° rapidly mounts to 110° or even 112°, followed by violent headache, maniacal or muttering delirium, unconsciousness and coma, which develops rapidly. The patient dies within a few hours of the onset of symptoms. Such cases may be mistaken for sunstroke or heatstroke.

Comatose.

(*b*) In comatose type the temperature does not usually rise above 104°, the patient, however, is apathetic from the beginning and rapidly becomes comatose. The coma may pass off with crisis of sweating, or an asthenic condition may set in and death from collapse may occur. The cerebrospinal fluid comes out under increased pressure and intracellular malarial pigment is a striking and diagnostic feature (Manson-Bahr). The large mononuclear cells are increased and there is a definite increase in the albumin and globulin content.

Other cerebral forms.

(*c*) In other cases delirium is the chief feature, and there may be mild excitement or a severe degree of mental confusion. The condition known as 'running amok' may be produced as a result of malaria. Convulsive seizures of an epileptic or tetanic type with or without delirium or coma, are specially common in children and are too often misdiagnosed, with fatal consequences. Forms resembling cerebrospinal fever, delusional insanity, acute alcoholism, and psychosis have been described. In some cases there may be mental changes of a permanent character. Syndromes of localised nervous involement, such as

aphasia, hemiplegia, monoplegia, multiple sclerosis, those of cerebellar or bulbar involvement or even encephalitis terminating with cerebral paralysis may be produced.

Algid malaria.

II. In algid malaria generalised depression predominates. The extremities and the skin are cold, the rectal temperature is high and there may be profuse sweating, nausea and vomiting; the face is pinched and the eyeballs sunken., the pulse is feeble and rapid, the blood pressure is low, the patient is collapsed as in shock (acute adrenal insufficiency).

The following are the principal varieties of algid malaria:—

Gastric form.

(*a*) In gastric type there is acute gastric irritation, *viz.,* severe epigastric distress, tender retracted abdomen and incessant vomiting which may contain blood.

Choleraic form

(*b*) Symptoms resembling cholera are produced, the stools suddenly becoming loose, profuse and numerous, but certain amount of bilious colouring being retained; the typical profuse rice water stools of cholera are absent and they may contain mucus even blood; dehydration with, pinched face, cramps in the limbs, aphonia, "washerwoman's fingers", and almost complete suppression of urine lead to a fatal collapse. "High axillary temperature if present, a recent history of intermittent fever with chill and rigor; the subsequent rapid cessation of choleraic symptoms on the appearance of hot and sweating stages; the colour of the stools, and other collateral circumstances usually suffice for diagnosis, particularly if they are supplemented by a microscopical examination of the blood. Although not usual, recurrence of the choleraic symptoms may take place at the next fever period". (Manson-Bahr).

Malarial Dysentery.

In severe subtertian malaria, gastro-intestinal disturbances, *e.g.,* diarrhoea with no fever and often ending fatally with symptoms resembling cholera have been observed. There may be no rigors or any of the ordinary signs of malaria. The stools become profuse, numerous, choleraic or mucoid with admixture of blood and ring forms of malarial parasites. Usually faecal matter retains some biliary colour but it may be colourless; dehydration occurs as in cholera. Diagnosis is made by making thick and thin blood films and looking for *P. falciparum,* Punctate haemorrhages occur in the mucosa, the vessels being full of parasitized cells; necrosis of the epithelium, leucocytic infiltration and even ulceration may occur. Sigmoidoscopic examination shows diffuse hyperaemia, swelling of the mucosa (as in bacillary dysentery) greyish yellow white patches show the presence of necrosis.

Treatment:—Cases of M.T. malaria with enteritis are very serious. The treatment should be started immediately with intramuscular injections of quinine (7 to 10 gr) and in more urgent cases intravenous injections should be given; after that a full course of mepacrine one tablet daily three times a day is given. To relieve intestinal symptoms, bismuth and magnesia mixture with opium may be given. If symptoms persist lavage of the bowel with a 2 per cent sodium bicarbonate solution or normal saline may be carried out.

Haemorrhagic form.

(*d*) In the haemorrhagic form, haemorrhages from the mouth, epistaxis, and bleeding from the gastro-intestinal tract, the nose, lungs, and female sexual organs occur. These symptoms have been frequently observed after administration of quinine or in quinine idiosyncrasy. The symptoms, however, in this type are cured by quinine.

Syncopoeal form.

(*e*) The symptoms in syncopoeal type show themselves in the stage of defervescence, there being excessive sweating leading to a fatal syncope. Weak and cachectic patients are more liable to such syncopoeal attacks.

Typhoid like malaria.

III. In typhoid remittent type, the onset is like enteric fever and the patient develops such symptoms as low delirium, prostration, dry tongue, enlargement of the liver and spleen. This type is frequently met with in India and many of such attacks prove fatal.

Bilious remittent malaria.

IV. In bilious remittent malaria there is high remittent fever, associated with obstinate vomiting of bilious material, jaundice, epigastric pain, and presence of bile in the urine. It may be confused with yellow fever unless a blood examination is made.

The other grave forms.

V. Other grave forms are classified according to the predominance of the symptoms of involvement of the different organs or systems.

Cardiac type

(*a*) Symptoms of myocardial failure with anginal pains due to interference with coronary circulation by infected red cells and various forms of disorders of cardiac action may be produced in malaria. Death from cardiac failure is common in pernicious forms of subtertian malaria and is due to the severe toxic fatty degeneration of the myocardium (Dudgeon and Clark).

(*b*) Symptoms resembling those of acute pancreatitis, acute appendicitis, ileus or intestinal obstruction or haemorrhage may occur as a result of malaria. *Acute abdominal type.*

(*c*) Signs of pulmonary congestion, bronchitis and mild bronchopneumonia relieved by administration of quinine have been reported; bleeding from the lungs most probably as a result of embolisation of small pulmonary vessels by infected red cells has been observed. *Pulmonary type.*

(*d*) The malarial attack may produce symptoms of haemorrhage nephritis. *Renal type.*

(*e*) Rapidly developing and progressive anaemia in the fourth or fifth week of a primary subtertian attack, comparable only to what is seen in the most advanced stage of pernicious anaemia is sometimes seen. There is great pallor of the mucous membranes and conjunctivae, cardiac distress and dyspnoea, haemic murmurs and retinal haemorrhages. Haemoglobin may fall to 10 per cent and red cells under 1,000,000 per cmm; leucopænia, nucleated red cells and microcytes may occur in the blood smear. The pathology of these condition shows the haemolytic nature of malaria toxin and its action upon the bone marrow which shows megaloblastic response (Fairley and Dew). *Hemolytic type.*

In subtertian malaria, mastitis is sometimes seen during the attack; orchitis has also been met with probably due to lighting up of an old gonococcal or tubercular infection. Haemorrhages in the female sex organs may occur. Dysmenorrhoea or even amenorrhoea may occur in untreated or imperfectly treated cases. *Other minor manifestations.*

(1). Chronic Malaria

Chronic malaria has been defined as a condition in which "as a result of repeated attacks of malaria, certain permanent changes are set up in the body. These chronic and permanent changes brand the patient as a sufferer from chronic malaria".

Chronic malaria may be produced in two ways:— (a) By relapses, that is to say by repeated exacerbations of a single infection; (b) by super-infection through which new parasites are added to the quiescent original strain. The first form is not common and is seen in person who protect themselves from the mosquitoes and who remove to a malaria free place after the first infection. The second form is commonly seen in endemic regions where little or no prophylaxis or treatment is undertaken. *Production.*

The clinical course is characterised by a large number of recurring attacks when a latent stage sets in. This is quite sudden in treated cases, but in others occurs only slowly, when the individual attacks become no longer fully developed and last for progressively shorter time. During this stage the patients develop anaemia, feel weak and complain of various troubles. This happens particularly when the primary attacks have been insufficiently treated. The patient has a characteristic pale complexion and there is enlargement of the spleen; lassitude, debility, diminution of sexual capacity in the male. *Clinical aspect.*

Examination of the blood shows the presence of gametocytes more frequently than schizonts, and leucopenia with relative monocytic increase. The urine shows excess of urobilin and urobilinogen.

Cases of chronic malaria often get relapses, from superinfection or from sudden lowering of vitality of the patient by cold, wetting, over-exertion, prolonged exposure to sunshine, alcoholic excess, mental excitement and strong purgatives. Relapses occur as a result of preventive inoculations with vaccines and with non-specific protein therapy, injections of serum and even organic arsenicals. Climatic variations like onset of spring and early summer may bring on a relapse.

The febrile attacks during relapses are like primary attack and in subtertian infection the second and third relapses may turn pernicious.

The chronic malarial infection, is characterised by attacks of pyrexia followed by latent period and relapses and latent periods go on alternating for a long time. Ultimately the periods of remission gradually become longer, relapses fewer and less severe, but vitality is lowered. Certain organic changes are characteristic of the chronic malaria:—In the blood (anæmia, leucopænia), the spleen (enlargement and fibrosis), the liver (enlargement with firm consistency), the skin shows pigmentation in dark brown irregular flecks common on the face, and folds of skin in the axilla and groin. The heart may show signs of dilatation, the cardiac rate is quickened and a systolic bruit may be heard, the pulse is

of low tension. A mild degree of albuminuria is not uncommon but true nephritis is rarely seen.

Malarial cachexia.

If chronic malaria is not treated and the disease allowed to develop unchecked, it passes on to malarial cachexia. The patient is anaemic and the complexion is dusky; œdema occurs round the ankles or may be more generalised. The abdomen is prominent (enlargement of the liver and spleen), the patient emaciated and asthenic, there is diminution of haemoglobin and red and white cells. Anisocytosis, poikilocytosis, polychromatophilia, punctate basophilia, nucleated red cells and relative increase of large mononuclears are present. The urine often contains albumin, and large quantities of urobilinogen and urobilin.

Other symptoms are visual disturbances due to intra-ocular haemorrhages, and malarial amblyopia. In children growth is inhibited. Adolescents show hypoplasia of genitals and retardation of onset of puberty. Adults may suffer from impotence and lack of sexual desire and rarely atrophy of the testes. Women have a tendency to sterility or miscarriage.

Certain mental changes are seen in malarial cachexia, *e.g.*, sensation of fatigue, dislike of mental effort and inability to concentrate, unreliable memory and occasionally aphasia.

In malarial cachexia, symptoms resembling beri-beri (cardiac weakness) may develop and gangrene of the extremities may set in severe cases. Liability to rupture of the spleen as a result of relatively mild injury is met with (Spleen puncture is dangerous). Schizogony is observed in peripheral blood without the usual paroxysms.

Masked malaria.

Masked malaria is usually manifested by such symptom as neuralgia of the trigeminal type, the attacks of which may occur regularly for sometime then disappear only to return later. Other symptoms are vomiting, migraine, attacks of tachycardia, herpes zoster, etc., The condition is amenable to quinine, though parasites may not be found; attacks of neuralgia due to other causes may respond to quinine.

Nervous sequelae.

Nervous sequelae of malaria are caused by long continued attacks and are seen in India. The sequelae include mental symptoms, aphasia, dysphasia and symptoms of disseminated sclerosis. Mental conditions that are sequelae of malaria are depression (melancholia and apathy) but confusional types may also occur; rarely states of elation, or excitement are produced. Caution has to be exercised before ascribing the symptoms to malaria and other concomitant malarial symptoms and signs of direct connection with previous attacks must be established.

Other so-called sequelae.

Numerous diseases, aetiology of which is still obscure or incompletely known, have been ascribed (specially by ex-service men seeking pensions) to be sequelae of malaria.

Leukaemia, pernicious anaemia, cirrhosis of the liver, myocarditis, arteriosclerosis, peptic ulser, chronic gastro-intestinal diseases, emphysema of the lung, pyorrhoea alveolaris, diabetes, nephritis, neurosis, epilepsy, have all been claimed as sequelae of malaria. Malaria has never been proved to be a cause of cirrhosis of the liver and other conditions are obviously not due to malaria.

(2) Malaria in Children

Congenital infection.

There are a few instances, reported by reliable workers, where infection in the infant came undoubtedly from the mother. Clark, studying the pathology of malaria infected placentas, found that although the maternal side of the placenta may show abundant plasmodia, the foetal side is entirely free in every case. He and others have shown that placenta is an effective barrier to the transmission of malarial parasites from the maternal to the foetal circulation and only some injury to the placenta, allows the parasites to reach the foetal blood. This also has been shown to be the case with monkey malaria. In *P. vivax* infection, Das Gupta considers that placental barrier is injured more by chronic rather than acute infection.

Incidence.

Children are extremely susceptible to malaria and in hyperendemic areas every child born in the area contracts malaria shortly after birth, and will go on contracting fresh infections.

Studies carried on by different workers in India have shown that practically 100 per cent of children born in hyper-endemic areas, upto the age of two years, are infected with malaria and have a high parasite count. These children are continuously febrile: what Christophers terms the 'stage of acute infestation'. After the age of three years the infection rate still remains as high as ever, but the average parasite count is relatively low. At the age of six to ten years the actual infection rate is still nearly 100 per cent, but the numerical

value of infections comes down to lower values. These children usually have attacks of malaria about once a month. In the same area only about 50 per cent of the adults are found to be infected and show a very low average parasite count. In adults, the attacks of clinical malaria are few and far between. The children are thus more frequently attacked by clinical malaria than the adults in hyper-endemic areas. Infant mortality from malarial infection is high in these areas, being almost double the figures in non-malarious localities.

Clinical aspects.

The clinical features of malaria in children differ considerably from those in adults. The individual attacks are irregular, the duration of the attacks may be so short as to escape notice; there may be no chill and rigor and the stage of sweating may be absent. The fever may come on only at night so that the pyrexial attacks go unobserved. None the less, the children progressively decline in health, and develop the features of chronic malaria and malarial cachexia; they are particularly liable to intercurrent infections which may be fatal. The acute attacks may be very severe giving rise to marked cerebral, gastro-intestinal, hyperpyrexial, or comatose symptoms, with fatal results.

Four types.

C. D. Williams has recently given an excellent description of the clinical features of malaria in children. (Lancet, March 9, 1940). In children four different clinical types of malaria can be distinguished. (*a*) Acute malaria with parasites present and distinct symptoms. (*b*) Chronic malaria with parasites present. (*c*) Chronic malaria in children from a malarious country, in whom no parasites can be found, but who improve under adequate treatment with quinine. (*d*) Presence of malarial parasites in the blood of children apparently normal and healthy in every way. These children are apt to develop clinical attacks whenever there is lowering of body resistance, *e.g.,* by exposure to chill, overwork, underfeeding, etc. These children are said to be more prone to suffer from intercurrent disease than children whose blood is free from parasites.

Symptomatology of malaria in children. Signs and symptoms may be absent or they may be so severe as to be fatal. Any of the following symptoms or any combination of them may be present.

Pyrexia.

Fever of 100°-103°F is perhaps the most usual symptom, rigors at the onset are not so common in infants as in adults. The fever may subside in a few hours and the child may appear quite normal between the attacks. Sweating during defervescence is also not so common in children as in adults. The temperature may be remittent in type in a fair proportion of cases, and the periodicity observed in malarial paroxysms of adults may not be well marked. Hyperpyrexia is rare in infants and less rare in older children, but if present it is often fatal.

Gastritis and Gastro-enteritis.

The gastro-intestinal tract of a child is more sensitive than that of an adult, and is much more liable to be upset by adversities, psychic, traumatic or infective. In an adult the attack of malaria may lead to little more than anorexia. In a child it may produce serious interference with digestion and assimilation, besides causing vomiting, diarrhoea or constipation.

Encephalopathy.

Children are apt to have convulsions at the onset of an illness, and the malarial paroxysm is no exception. Convulsions often replace the rigor at the onset of malaria in children. These often take the form of clonic or tonic contractions, though they may be of an epileptic type.

Cerebral symptoms at the onset of malaria, however, do not necessarily mean cerebral malaria, but the two are difficult to distinguish and it is as well to regard malaria accompanied by convulsions as very serious, although many patients recover.

Splenomegaly

The lymphoid tissue in a child is exceedingly labile compared with that of an adult. The spleen of an infant 6 months old may, in response to an attack of malaria, enlarge from the costal margin down to the umbilicus in four days and with adequate treatment return back to normal in a week (unlike fibrotic spleen of chronic malaria).

Hepatomegaly & anaemia

Enlargement of the liver is often a prominent feature of malaria in children, and often accompanies the enlargement of the spleen. It is as well to recognise this sign of hepatic damage before jaundice, which may be quite severe and last long. Anæmia is caused by destruction of the red cells; there may be some associated toxic process the nature of which is not understood, in the causation of such anaemia in children. Traces of albumin may be found in the urine of children with malaria and are generally not of much importance.

Cachexia

Malarial cachexia is by no means uncommon in children especially in those older than 9 months. These children are lethargic, querulous, and apathetic, with a grey unhealthy look. The children become thoroughly run down and anaemic and there is considerable enlargement of the spleen and the liver, causing protuberence of the abdomen.

Blackwater fever is almost unknown in infants, rare in older children, and relatively uncommon in adults born in a malarious area.

Prophylaxis and treatmcnt.

Quinine atebrin, paludrine and chloroquine are useful as prophylactic agents.

Dosage recommended to be taken daily at bed time.

Age	Dose	Age	Dose
Quinine:—			
Birth to 9 months	gr. i	2-6 years.	gr. iii
9 months to 2 years	gr. ii	6-14 „	gr. iv

Quinine.

Atebrin:—0.025 to 0.05 daily or 0.1 gm or twice weekly according to age.

Paludrine:—50 to 300 mg daily according to age for curative therapy and 50 to 100 mg once a week for casual prophylaxis.

Treatment.

The general principles to be followed in the treatment of malaria in children differ but little from those in the case of treatment of malaria in adults. Treatment should aim at elimination of the parasites, but where the child has to remain in malarious country after cure, and thus is certain to be reinfected, therapeutic measures directed to cure of the symptoms of an attack are all that is practically useful. Besides, the complications and sequelae of acute attacks *viz.*, anaemia, cachexia, hepatitis, etc. have to be treated.

Quinine has been found to be quite satisfactory drug in treatment of malaria in children. The only disadvantage are its unpleasant taste, difficulties of absorption and some undesirable after effects. Quinine ethylcarbonate (Euquinine, is mostly inactive and is not advised). The fever itself might lead to diminished absorption of quinine. Also helminthic infection or other gastrointestinal disorders would do the same, so that oral medication may fail in its objective. Hence parenteral medication has to be considered.

Parenteral administration of quinine is the method of choice in severe cases, in cases that do not react to quinine given by mouth, and those in which the parasites persist inspite of treatment, and in cases where gastro-intestinal symptoms are such as to preclude the adequate absorption of quinine given orally. Intravenous injection of quinine is usually very difficult to administer in children and is not free from risks. The intramuscular route has to be preferred.

Quinine by mouth. The following prescription is advisable:—Quinine sulphate 2 grains, ferrous sulphate 1 grain, acid sulphuric dilute 4 minims, and water up to one dram. For a baby of one month old the dose is one dram thrice daily for older children correspondingly increased doses are given. This dosage will relieve fever and cause disappearance of parasites in a week. Severe cases and the cases where quinine is not being absorbed should be treated by quinine parenterally.

Atebrin.

Children tolerate atebrin well. Dose, 1 to 4 years 0.05 to 0.1 gm; 5 to 10 years, 0.2 gm; and over ten years, 0.3 gm daily. It can also be given intramuscularly in doses of 1 ccm up to two years of age, 2 to 4 ccm upto 12 years, of a 0.3 gm in 9 ccm solution.

Paludrine. The lack of bitter taste and its extremely low toxicity make paludrine a very useful drug for use in children. It may be given in doses of 50 mgm for children below 2 years of age, 100 mgm daily for children below 10 years of age and 300 mgm daily for children over 10 years.

Treatment of complications. Convulsions should be treated with sedatives, by chloroform inhalations if necessary, and lumbar puncture. A hot bath with ice cap to the head is often useful. Anaemia is treated with ferrous iron and adjuvants. In cases with severe anaemia transfusion of blood is indicated. The transfusion is made through the vein in the anterior fontanelle if necessary.

Blackwater fever is treated as in adults. Malaria in children is as amenable to atebrin therapy as in adults. The details of dosage have been given under Atebrin.

16. Black-Water Fever

(1) General

Haemoglobinuria which occurs as a symptom of malaria is called black-water fever. It is precipitated by quinine and other drugs, but is essentially a severe manifestation of malaria or one of its outstanding sequelae. The distribution of the disease runs parallel with that of severe malignant tertian malaria.

Although the ætiology of blackwater fever is still undecided there is general agreement that malignant tertian malaria is always associated as a predisposing cause. It is a complication of malaria, occurring mostly in hyperendemic areas. Blackwater fever occurs in Central Europe, from Spain to the Balkans; in Turkey and Palestine; in such malarious part of India as Assam, Bengal and the Dooars; throughout the tropical and sub-tropical parts of Africa from Algeria to Abyssinia and Rhodesia; Mauritius, Madagascar, the Southern United States, the West Indies and South America. The disease is very rare in mountains, but is common in the country and forest areas. It tends to recur in the same houses when they are near the breeding grounds of anophelines. Malarial parasites occur in over 73 per cent of cases, generally *P. falciparum* is present but the infection may be mixed. *P. vivax* and occasionally *P. malariae* have also been found. One attack of blackwater fever predisposes to another attack so that as many as 13 attacks have been recorded but without any tendency to increased severity of the later attacks. The simplest and most widely held theory about its causation is that as the result of repeated destruction of red blood corpuscles owing to recurrent attacks of malaria, a hæmolysin is formed and hæmolysis is produced in the same way as by the injection of foreign blood into animals. It is asserted that the products of chemical changes brought about in the corpuscles due to the presence of the plasmodia act like a foreign material. No hæmolysin has however been demonstrated in the blood.

The exact mechanism involved in the hæmolytic process is still obscure, but the likely explanation is that in certain circumstances the red cells become sensitized to hæmolytic substances produced by the malarial parasites directly or by their action on the red blood cells. The rapid hæmolysis appears to be a phenomenon of a type similar to allergic or anaphylactic shock.

Pathology.

The sudden hæmolysis of large numbers of red cells sets free large amounts of hæmoglobin. The cause of hæmolysis is not known. There is no increased fragility of erythrocytes, nor is there evidence of autohæmolysis; no hæmolysin has been discovered. A part of hæmoglobin is taken up by the reticulo-endothelial cells and the remainder is excreted by the kidneys, the cells of the renal tubules being damaged (granular degeneration) in the process. In some fatal cases the tubules may be completely blocked by the degenerated cells and debris, so that anuria is produced. In less severe cases the urine contains large amounts of albumin, granular debris and tubular casts. In the presence of acid urine, acid hæmatin is deposited in the tubules producing renal insufficiency and azotaemia. The red cells may be diminished by 2 or 3 million per cmm within 24 hours.

The pigment in the plasma was believed to be methaemoglobin but it has recently been shown to be methæmoalbumin (pseudomethaemoglobin) an abnormal product derived from oxyhaemoglobin liberated from red cells. Hyperbilirubinaemia and jaundice occur in all cases and indirect van den Bergh reaction is positive. The red or black colouration of urine is due to oxyhæmoglobin, methæmoglobin and urobilin.

Post-mortem findings are those of severe hæmolysis and malaria.

Clinical aspect.

Symptomatology—The onset is sudden with rigor and rapid rise of temperature. The fever is generally of a remittent type and may last from a few hours, up to 6 days or more. Three cardinal symptoms are hæmoglobinuria, fever and jaundice.

In the mildest type there is slight fever and slight haemoglobinuria lasting a few hours, and the attack may be difficult to distinguish from one of ordinary malaria. In less milder types, the fever and hæmoglobinuria may last for one or two days, and the general condition of the patient is not seriously affected. In moderately severe types the fever lasts 3 or 4 days with pronounced jaundice and haemoglobinuria. In the severest type, and nearly all fatal cases are of this form, the symptoms are severe from the onset, and suppression of urine, extreme anaemia, heart failure collapse, and hyperpyrexia, are prominent features. Occasionally some attacks are relatively mild at first but repeated paroxysms result in rapid deterioration of the patient's condition.

Vomiting is usual, and there may be epigastric pain or severe lumbar pain. Jaundice may be moderate or intense and appears early and lasts a few days after the fall of temperature.

The urine is pink or bright red at first, but soon becomes dark red, brown or black. It is loaded with albumin and debris and tubular casts are numerous.

The pulse is of low tension, weak and rapid. There is a feeling of prostration with anxiety and restlessness but the mental condition is usually clear. Heart failure due to toxaemia and severe anæmia is a frequent cause of death.

Diagnosis.

The majority of cases occur in areas where the disease is well known and diagnosis presents no difficulties. The disease must be distinguished from hæmaturia, in which case microscopic examination of the urine will show numbers of red cells. In malaria with bile-stained urine, a piece of blotting paper dipped in the urine, is stained yellow or greenish yellow, whereas in haemoglobinuria it is stained red. Paroxysmal hæmoglobinuria and quinine haemoglobinuria are rare conditions. In yellow fever and leptospiral infections, the condition is one of haematuria and not hæmoglobinuria.

Prognosis.

Prognosis is always grave but it depends on the severity of the attack, the age and general condition of the patient. The average mortality ranges from 25 to 50 per cent in various localities. Hyperpyrexia, deep jaundice, severe anaemia, hiccough, and persistently recurring paroxysms are unfavourable features as also is rapidly rising curve of blood urea.

(2) Treatment

General.

The general management of a case of blackwater fever is the most important aspect of the treatment. Absolute rest in bed, avoidance of chilling, and good nursing are essential. The patient should be kept warm and should not be moved after the disease has set in; the mortality rate is increased by transportation of patient. He should not be allowed to sit up in bed; the writer has seen fatalities occuring just by the exertion of sitting up in bed. The patient should not be allowed to get up untill temperature has remained normal for several days.

Fluid diet.

Administration of fluid by mouth, per rectum or even intravenously is an essential part of treatment. Water containing 30 grains of sodium bicarbonate to the patient should be given frequently and the urine should be kept alkaline in reaction. Albumin water or barley water are retained better when given by mouth; also glucose. Small quantities should be taken at a time. In serious cases, saline or glucose saline may be administered per rectum or intravenously. As haemoglobinuria may recur on full diet, it is advisable to carry on with milk, broths, and fruit juices well into the convalescence.

The daily fluid intake should be 2000 to 6000 ccm per day and the actual intake should be so adjusted that 1500 to 2000 ccm of urine is passed. The bladder should be emptied every four hours with a catheter if necessary, and its reaction should be tested. If during the pre-blackwater stage, general and alkali treatment is instituted, mortality is considerably reduced. A mild saline cathartic should be given at once. The consensus of opinion is that the action of atebrin

Qunine.

in influencing the onset of blackwater fever is the same as quinine. As a large number of parasites have probably been destroyed by general haemolysis antimalarial drug may not be necessary in the first day or two of disease. If haemoglobinuria occurs in the course of a malaria paroxysm and parasite are present in the blood, quinine or atebrin may be given. If parasite are not found in the blood these drugs should not be given. If quinine has already been given before haemoglobinuria and there are no parasites, quinine should be stopped; if however parasites present, it should be continued. In patients who have an attack of blackwater fever quinine should be given with caution and in exploratory doses. Every black-water patient has a critical threshold dose of quinine.

Atebrin and plasmochin.

Many authorities believe that fear of giving quinine in blackwater fever is exaggerated. Quinine should, however, be given cautiously so as not to produce nausea and vomiting. Maegraith (1946) considers that there is no evidence that mepacrine is haemolytic and recommends its use in full doses. Blackwater-fever has, however, been reported in malaria treated with mepacrine alone, and it may be better to use paludrine when malarial parasites persist during such an attack.

Plasmoquine is definitely contraindicated and so is its combination with atebrin. If plasmodia occur during convalescence, etc., atebrin may be given 0.1 gm on first day, two doses of 0.1 gm on the second day, and three on the third, fourth and fifth days. This is the drug of choice in blackwater fever.

Alkalis.

Hot fomentations to the loins relieve the pain by removing renal congestion. There is clinical and experimental evidence to suggest that suppression of urine is much less likely to occur if the urine is alkaline; therefore sodium citrate and sodium bicarbonate in adequate doses should be administered to keep the urine alkaline, and in severe cases alkalis may have to be given intravenously. An alkaline solution containing sodium chloride 5.75 gm, sodium bicarbonate 18.25 gm and distilled water 1000 ccm should be given every 12 hours, if vomiting is severe, urine is acid in reaction or there is anuria. If urine remains acid give 15 to 20 grams of sodium bicarbonate by the mouth every two hours till it is alkaline. A 5 per cent solution of glucose in warm saline has been recommended, one pint at a time. Larger quantities of fluid by intravenous route are not advised because these may produce œdema of lungs. Others advise 150 grains of sodium bicarbonate in a pint (500 ccm) of distilled water. High rectal enema of hot water or saline may stimulate diuresis. Dry cupping over the loins and caffeine citrate 2 gr. a day are other methods to overcome threatened urinary suppression.

Whole blood transfusion.

Transfusions of whole blood may be given when there is rapidly developing severe anaemia. Washed, packed red blood corpuscles suspended in normal saline have been recommended. Blood transfusion is especially useful in toxic and relapsing type of cases, but it is contraindicated in toxic and anuric blackwater fever. Care should be taken in grouping the bloods as auto-agglutination may occur in haemolytic conditions. The blood may be given as citrated whole blood (500 ccm) every 24 hours or even twice a day in serious cases. The patient should be carefully watched while the transfusion is being given; if any marked reaction occurs O.5 ccm of adrenaline may be given intramuscularly.

Plasma Therapy.

When proper cross-matching and typing of the blood is not possible, blood plasma should be used in which case matching is unnecessary. Plasma is valuable in combating shock and keeping up the strength of the patient; one pint (500 ccm) transfused twice daily, reduces mortality considerably.

Vomiting may be alleviated by application of turpentine stupes and mustard plaster to the epigastric regions. Morphia in very small doses may have to be administered to allay restlessness. Antipyretics are contraindicated because of their depressing effect on the heart.

Specific drugs used are cholesterin and cortin; 15 grain doses of cholesterin administered in thick suspension or in milk four hourly act as anti-haemolytic. Cortin is believed to counteract degenerative changes in the adrenal cortex. Cholestrin may also be given intramuscularly in warm olive oil (5 per cent solution).

The convalescence should be prolonged and gradual, and return to normal diet and life very slow, because otherwise there is great tendency to relapse. Patients who have previously had an attack of blackwater fever should not be allowed to return to a country where malaria is prevalent. Such patients should also not be transported suddenly from a hot to a cold climate.

Prophylaxis:—All cases of malaria, especially *P. falciparum* infections should receive adequate drug treatment. If malarial prophylaxis is properly carried out, blackwater fever does not occur. This has been amply shown during World War II. If there has been a previous attack, atebrin or paludrine is preferred to quinine. Exposure, chills, over-exertion, dietary errors, alcoholic excesses and other conditions leading to lowering of vitality should be avoided.

Pre-blackwater state should be recognized. This consists of toxaemia, slight jaundice, enlarged and tender liver and dark urine. If this occurs, antimalarial therapy with complete rest and alkali treatment, should be instituted.

17. Induced Malaria

(1) General

Von Jauregg (1877) first drew attention to the favourable effect of malaria on certain mental disorders but systematic clinical trials were not began till 1917. For artificial induction of malaria in man, two methods have been employed:—

Two methods of induction. Inoculation of Blood.

(1) Inoculation of blood containing malarial parasites, either subcutaneously or intravenously. The amount of blood required for this purpose varies from 0.5 to 5.0 ccm. For intravenous injection 0.5 to 2.0 ccm of blood suffices, but for subcutaneous inoculation 3 to 5 ccm may have to be given. Malarial blood can be transported long distances for this purpose, but it has to be defibrinated or mixed with a small amount of sodium citrate before it is sent; it remains quite effective for 48 hours if it is kept in a cool place. The incubation period is shorter after intravenous than after subcutaneous injection, depending on the number of parasites contained. If there has been previous exposure to the disease the incubation period is longer. The stage of fever of the donor at the time the blood is taken is also important; if blood is obtained during the relapse period, it is much less virulent than if it is taken during the course of an attack. The period of incubation in malaria induced in this way averages between 4 to 10 days in case of intravenous injection, but it may take as long as 50 days in case of subcutaneous injection.

Infection through Mosquitoes.

(2) By means of infested mosquitoes, the sporozoites being introduced by the bite of the mosquito. The sporozoites isolated from the mosquito may be injected. In England *A. maculipenis* is generally used; *P. falciparum* will not develop in this mosquito at low temperatures. It is advisable to use only *P. vivax* for inducing malaria. In endemic areas in India, the writer has rarely found great difficulty in inducing of malaria on account of immunity which has been produced in these patients. In such cases blood containing *P. falciparum* has been used, the patient being very carefully watched and frequent blood examinations being made. No untoward effects were encountered. The danger of mixed infections is always there especially in India.

A. bifurcatus has also been employed. The virulence of the infection produced varies in different individuals from a very mild to very severe infection which may end fatally even with B.T. infections. Daily examination of blood should therefore be made and treatment should be started as soon as warning of danger is received. Induced malaria does not differ essentially from natural malaria, the kind of plasmodium of the donor determines the type of fever induced. Inoculated malaria may take the tertian type, but often it is of quotidian type. An initial fever often occurs before intermittent attacks but this may be absent in second inocculation or in immune people.

Application in neurosyphilis diseases.

In the treatment of cases of dementia paralytica, usually 8 to 12 attacks are allowed, and in case of quotidian as many as twenty, the writers' experience in India is that it is generally not advisable to give more than six to eight paroxysms. Spontaneous cures rarely occur after the first inocculation and often only after numerous attacks. Reinoculation is possible after recovery but if the same strain of parasite is used it may take several weeks for the fever to appear and then it may be mild or it may not develop at all. This shows that immunity has developed.

Rarely first inoculation may fail and a second or even a third may have to be given, and if that fails, infection through mosquitoes may have to be tried.

Symptoms and course.

The symptoms produced are generally mild, but the induced B.T fever should not be considered harmless. The attacks should be cut short atonce if severe anaemia and jaundice appear and the number of parasites in the blood is large. Rarely rupture of spleen has occured.

Transmissible gametocytes appear, though in smaller numbers than in natural malaria. Susceptibility to mosquito-borne infection was retained even after fifty four inocculations in the course of 3½ years (Yorke & Wright). The essential difference between inocculation and natural malaria is the absence of relapses in induced malaria and this is one reason why conclusions drawn from induced malaria cannot be applied to natural malaria so far as treatment, prophylaxis and immunity are concerned. Malaria transmitted by mosquitoes, runs more like a natural course and has the usual relapses (James, Korteweg).

(2) Treatment

Infection produced by direct inocculation of blood is easy to cure permanently as there are no relapses. Such small doses as 3 to 10 grains of quinine often stops the fever permanently in case of Indian strains, the relapse rate being only 2 to 4 per cent. In other cases 0.6 to 1.0 gm of quinine or a few days course of atebrin will cut short the fever permanently. Some strains e.g., Madagascar strain react less to quinine and better to atebrin and neosalvarsan (Nocht & Mayer). Occurence of blackwater fever has been observed in induced malaria especially after quinine, but in the writers experience in Calcutta it never occured.

Effects on general Paralysis.

Fever and high temperature probably act by their deleterious effects on the spirochaetes responsible for the disease and similar effects have been observed with non-specific protein therapy (bacterial vaccines) and pyrogenic drugs. Brutsch and Bahr have demonstrated proliferative changes in the capillary endothelium in the brain substance after induced attacks. These must be regarded as local reaction of the reticulo-endothelial system; perivascular infiltration occurs. After these changes resolve, partial regeneration of ganglion and neuroglia cells may occur.

When such treatment is being carried in the paralytics, they must be protected with wiregauze mosquito netting to prevent mosquitoes being infected and transmitting infection to healthy individuals.

18. Antimalarial Drugs in Simian Malaria

A few words may be said about the action of these drugs on ape-malaria. It has been shown that *P. knowlesi* produces a very intense and virulent infection in *Silenus rhesus*, causing death of the animal if untreated. This plasmodium appears to be more closely

related to that occurring in man than plasmodia occuring in birds, which are usually used for testing antimalarial drugs. The results obtained should therefore be more readily applicable to man. Chopra and Das Gupta (1933) showed that the destructive action of atebrin on *P. knowlesi* was exceptionally powerful. Usually two doses of 0.025 gm of the drug given intramuscularly or intravenously are sufficient to control a very heavy infection which may amount to a million parasites per cmm of blood. The drug affected equally the schizogony and the gametogony. Owing to its slow excretion, atebrin appears to exert a more prolonged action than quinine and atebrin is successful in checking a heavy infection whereas quinine may not. It was, however, shown that even after five days intensive treatment with atebrin, the parasites invariably reappeared in the monkeys in 10 to 15 days and multiplied with the same rapidity as in the previous attack, causing death of the animal if prompt treatment was not given. The recrudescence can, however, be checked more easily, one dose sufficing to control the multiplication of the parasites. After this a low grade of infection persists for long periods, the parasites losing their virulence. In contradistinction to the action of atebrin, quinine, according to Chopra and Das Gupta (1934), has a much less powerful immediate effect on these parasites. When the parasite count is low, *i.e.,* below 100,000 per cmm, one dose (intramuscular or intravenous) may be effective in controlling the infection, but when the parasite count is higher, 2 or 3 injections are necessary to control the infection. Further, the first effect of injection may be an actual increase in the number of parasites. The advantage of quinine is that relapses rarely occur after the treatment of the primary infection, and even when they do occur, they are not often fatal. The action of quinine thus appears to be slower and if the treatment is started at a time when the infection is moderately heavy, the infection does not seem to be effected, in most cases, in 24 hours or even longer with quinine, whereas with atebrin even if treatment is started late, *i.e.,* when the count exceeds half a million parasites per cmm., this invariably falls to a negligible number.

Stimulated by the results of these experiments, further work has been carried out on the concentration attained by these drugs in the circulating blood at different intervals of time in relation to parasite count. With regard to quinine, Chopra, Ganguli and Roy (1935) have shown that there is no direct relationship between the concentration of quinine in the blood and the parasite count at any particular time. The highest concentration of the alkaloid attainable without producing toxic effects produced no apparent reduction in the number of parasites nor degenerative changes in them. The action of quinine thus appears to be synergistic to other defensive mechanisms set up in the body. In therapeutic doses quinine augments these processes or possibly it acts on the parasites in such a way as to render them more vulnerable or unable to propagate. Studies on the concentration of atebrin in the blood by Chopra, Ganguli and Roy (1936) show that the highest concentration of atebrin occurs between half an hour and six hours after the injection of atebrin. The number of parasites per cmm of blood distinctly diminishes in the majority of cases in the first 6 hours when the concentration of atebrin in the blood is highest. Within 24 hours the parasites are reduced to a negligible number even in spite of the concentration rapidly falling off. It is, therefore, reasonable to state that atebrin unlike quinine, has probably some direct lethal action on *Plasmodium knowlesi* in vivo. This has been further confirmed by Chopra, Das Gupta and Roy (1936) who showed that atebrin solution in a dilution of 1 in 50,000 *in vitro* is capable of destroying the parasites even when the infection is heavy. The smears of blood which were kept in contact with atebrin showed degenerative changes in the parasites. This work is being further elaborated.

Three important points which come out of this work are:

(1) Atebrin has a more powerful and more rapid effect on *P. knowlesi.* Whereas quinine takes 24 hours to produce its effect, atebrin is effective in 6 hours and concentration of this drug runs parallel with the decrease in the number of parasites.

(2) Atebrin has some direct action on *P. knowlesi* (1 in 50,000 concentration), quinine has not. In fact the first effect of quinine administration may be an actual stimulation of growth of parasites.

(3) A fatal relapse is more common after atebrin than after quinine. The action of quinine, though less powerful and less rapid, appears to be more prolonged and lasting. Whether all these are applicaple to human malaria remains to be seen.

19. Present Position of Antimalarial Drug Therapy

The present position of antimalarial drug therapy may now be summarised. The following groups of drugs are used in the treatment of malaria:—

(1) Cinchona alkaloids.

(2) Synthetic antimalarials

(*i*) Plasmochin and allied compounds—The anino-quinoline derivatives.

(*ii*) Atebrine and similar compounds—Acridine derivatives.

(*iii*) Sulphonamides.

(*iv*) Paludrine.

(3) Cinchona Alkaloids Versus Synthetic Antimalarial Drugs.

(1) Cinchona Alkaloids

The importance of the fact that all alkaloids have marked antimalarial properties is very great to India where the economic condition of the pepole is low and purified quinine preparations, on account of their high price, are beyond the means of ordinary people. The reasons why quinine is more expensive than the total alkaloids of the bark are:—(*i*) that its proportion in the bark usually is a little more than half of the total alkaloids. *Cinchona succirubra* and *Cinchona officinalis* barks, for example, contain about 5 to 6 per cent of total alkaloids of which only 3 per cent is quinine. *Cinchona ledgeriana* contains somewhat higher proportions. (*ii*) The cost of its separation from other alkaloids and purification further add to its price. It would follow, therefore, that if the total alkaloids are used they will be cheaper than if only quinine is used by itself. It was for this reason that 'cinchona febrifuge' was used in India and the League of Nations have introduced *totaquinina* which contains 70 per cent of the total alkaloids of which 15 per cent must be quinine. By this means it is hoped that the treatment could be made less expensive and extended among the masses.

Some of the other alkaloids of the cinchona bark which occur in smaller quantities than the four alkaloids stated above, for example cupreine sulphate in doses of 1 gm, was found to be effective in human malaria, but it is much more expensive and toxic. The same is the case with other such alkaloids.

The disadvantages of the cinchona alkaloids are:—

Disadvantages.

(1) They have no action on the sporozoites injected by the mosquitoes and therefore they cannot have true prophylactic action in this disease. This was demonstrated by Yorke and Macfie (1924) who showed that 18 grains of quinine daily for 5 days before and 7 days after the mosquito bite failed to avert an attack of a malaria, but if the drug was continued for 10 days, the disease did not develop. This shows that these alkaloids have little effect on the injected sporozoites but act on the asexual forms liberated from the infected red blood corpuscles.

(2) Cinchona alkaloids have little effect on the sexual forms of malignant tertian parasites. They however, impede the formation of the pre-gametocytes.

(3) They do not prevent relapses.

(4) These alkaloids do not act uniformly on all strains of malaria.

After the World War I there was a feeling against the use of cinchona alkaloids especially quinine and it was said that this drug was not effective against certain forms of malaria. Further investigation, however, showed that this was not due to any fault of the drug but due to its improper use.

It has been proved by hæmatological and other evidence that the best method of administration of these alkaloids is by the mouth. It is advisable to give them in the form of solution but owing to their bitter taste they may produce vomiting. They can be given in the form of tablets so long as these are easily disintegrable. Subcutaneous injection finds few advocates. Intramuscular injection should only be tried in those cases where administration by the mouth is not desirable or possible as it is liable to produce serious mutilation of parts. Intravenous method should only be used for severe and urgent cases. Parenteral method should not be used for routine administration of these drugs. Intravenous injections must only be employed where the patient is comatose, or very gravely ill when an immediate response is necessary. Any evidence of impending cerebral malaria is an imperative indication for injection treatment.

On the appearance of acute symptoms of primary infection, quinine has a definite action from the third day onwards (second paroxysm of fever) in benign tertian malaria. Its action is less rapid according to the strains of malignant tertian concerned and symptoms may continue until the fifth dose (third or fourth paroxysm).

As regards the time of administration of these alkaloids by the oral route, it is advisable to give them 2½ to 3 hours after a meal when the gastric contents are acid, the digestion has been complete and the stomach is nearly empty. If given at this time they rapidly mix with the contents of the stomach and pass into the small intestine from where they are quickly absorbed into the circulation. There is less liability of their irritating the stomach if administered in this way.

Dosage.—Experience shows that in the case of Indian strains of malaria, a 7 to 10 days' course of treatment with 20 grains of the alkaloids daily is effective. Larger doses are not only wasted, but may do harm. According to the Fourth General Report of the League of Nations, 0.5 gm of quinine hydrochloride sometime causes a temporary disappearance of trophozoites of benign and quartan infection but a mean daily dosage of 1 gm for 5 to 7 days is often necessary to cause the trophozoites to disappear and not to make their reappearance in the peripheral blood after a latent period of varying lengths, in the course of first relapse. In malignant tertian malaria 1.3 gm produces analogous results. It is also agreed that definite advantage is obtained by combining them with alkalies.

Proprietary preparations.

Preparations containing Cinchona derivatives. During recent years a number of cinchona derivatives have been used in the form of proprietary remedies whose composition is secret. Various claims have been made for these, amongst others that they have destructive action on both the sexual and asexual forms of all parasites, that they prevent relapses and that they have prophylactic value. A number of preparations are on the market and the writer has tested some of these. Our observations show that their action is practically the same as cinchona alkaloids, they have no action on the gametocytes of malignant tertian, they do not prevent relapses. Being proprietary remedies they are very much more expensive. Some of these preparations in the market are:—*Malarcan,* probably a derivative of quinine or quinidine, *Tebetren* (quinine combined with acridine), *Paludex* (complex organo-metallic preparation containing copper and oxyquino-

line), *Plasmodex* (a similar compound), and *Esanofeles* is a proprietary preparation containing 0.1 gm of quinine bisulphate, 0.001 gm of arsenious acid, 0.3 gm of iron citrate and some bitter principles. It is used in India by medical practitioners in chronic malaria.

(2) Synthetic Antimalarial Drugs

Groups according to activity.

These compounds may be divided into three groups:—(1) Those which have action like the cinchona alkaloids on the asexual cycle, *e.g.*, atebrin and atebrin like substances, sulphonamide derivatives, etc. and (2) those whose main action is on the sexual cycle particularly of the malignant tertian variety, *e.g.* plasmochin. (3) Those which act on the extra-erythrocytic phase *e.g.*, paludrine, pentaquin, etc.

None of these compounds act on the sporozoites injected by the mosquitoes and therefore have no true causal prophylactic value except perhaps paludrine in a limited degree because of its action on exo-erythrocytic cycle.

Their dosage as compared with the cinchona alkaloids is much smaller, but they are usually more toxic.

(i) The amino-quinoline derivatives

Effectiveness.

(*a*) *Plasmochin* (*Pamaquin*).—The shortage of quinine supply during the war led to experiments in Germany for finding a synthetic drug which could be used in its place. Schulemann and his colleagues chose methylene blue for investigation; they prepared a large number of compounds which were tested on canaries infected with *Plasmodium relictum*. Among these compounds they found one which was particularly effective and this was an amino-quinoline derivative in which a basic aliphatic radicle was united to a quinoline nucleus by a connecting link of nitrogen. This compound was modified in many ways, and eventually the compound, first known as 'beprochin' and afterwards as *plasmoquin* or plasmoquine or *plasmochin* was produced. It was at first thought to have a definite curative effect in the treatment of all forms of malaria, but later experience showed that, while it was effective in adequate doses (which often produce toxic effects) in curing the benign tertian and quartan malaria by itself, in sub-tertian malaria it was of little therapeutic value because it had no action on the schizonts of *P. falciparum*. It, however, possesses the remarkable and unique property of destroying the crescents in the peripheral blood.

Toxicity.

On account of this peculiar property of damaging the gametocytes it renders them non-infectious to mosquitoes. Even such doses as 0.02 gm of plasmoquin daily are effective in this respect. This remarkable effect against the more resistant form of the parasites is the greatest virtue of the drug but unfortunately it has little or no effect on the asexual form of malignant tertian parasites. Plasmoquin is a drug which is likely to give rise to toxic effects and it should not be used for routine treatment of malaria. It is combined with quinine, and it is claimed that this combination when continued for 2 to 3 weeks is more effective than quinine and the number of relapses is decreased. Prolonged administration of this combination in therapeutic doses is likely to produce toxic effects but these are not so marked as a combination of plasmochin with atebrin. Plasmoquin by itself thus holds a minor place in the symptomatic treatment of malaria. Cilional and Certuna are similar drugs, but are not so effective.

Quinine & plasmochin. It has been found in India that a combined course of quinine and plasmochin (pamaquin) was more effective in preventing relapses in benign tertian malaria than quinine alone. Ten grains (0.65) of quinine and 10 mgm of plasmochin three times a day for ten days were recommended. Later investigations confirmed these conclusions and found that during the six months following a combined course only 10 per cent of cases relapsed as compared with 30 per cent treated with mepacrine alone. The combination of quinine and plasmochin is, however, more toxic than either of plasmochin and mepacrine.

(*b*) *Chloroquin.*—(SN 7618) 7-chloro-4-(4-dimethylamino-1-methylbutylamino) quinoline. This compound was used by the German forces in North Africa as an antimalarial drug during World War II. It had been prepared by the I. G. Farbenindustrie and was known as Sontoquin. Its composition was worked out and its derivatives were prepared in the United States. One of these—chloroquin—on clinical trials was found to be better than mepacrine—as curative and suppressive drug in malaria. Its action resembled paludrine in completely curing malignant tertian malaria, but although it controlled the clinical attacks of benign tertian, it did not prevent relapses. The advantage which it possesses over mepacrine is that it dose not stain the skin yellow.

The following doses are recommended for the treatment of all forms of malaria: Curative 0.6 gm. of chloroquine base (1.0 gm. of chloroquine diphosphate) on the first day followed by 0.3 gm. of base in 6 hours; for the next two days 0.3 gm. daily. The total amount given is 1.5 gm. of the base or 2.5 gm. of diphosphate salt in 4 days. The parasites disappear from blood within 24 hours. For suppression purposes 0.3 gm. (or 0.5 gm. of the salts) once a week is used for adults and for children under 8 years 0.15 gm. of base (or 0.25 gm. of the salt). The drug is usually given in tablet form.

Toxic symptoms consist of mild headache, blurring of vision, dizziness, diarrhoea, pruritis, urticaria, etc. The toxic symptoms are controlled by giving ammonium chloride to make urine acid.

(*c*) *Pentaquin.*—(SN 13.276) 6-methoxy-8-(5-isopropylaminoamylamino) quinoline. Both paludrine and chloroquin do not cure benign tertian malaria and the problem of finding a drug which destroys all forms of parasites in the body still remained. Sinton showed that when quinine and plasmoquin (pamaquin) were given together in benign tertian malaria, the relapse rate was lower than with quinine or mepacrine alone. Compounds allied to plasmoquin were then studied in the United States and pentaquine was produced. In both these compounds the ring-structure is methoxy-quinoline. The same side-chain is present in mepacrine, plasmoquine, and chloroquine.

The advantage of pentaquin is that it is much less toxic than plasmochin in bird malaria but a daily dose 0.06 gm. of pentaquin is more toxic than 0.03 of pamaquin. It is the most active compound yet discovered against bird-malaria. In volunteers infected with virulent type of *P. vivax,* who were treated with full doses of quinine and pentaquin the infection was completely eradicated.

Dosage. 0.01 gm. of pentaquin base (0.013 gm. of mono phosphate salt) is given with 0.6 gm. quinine sulphate three times a day for 14 days, making a total of 0.42 gm. of pentaquin base and 28 gm. of quinine sulphate. Toxic symptoms are similar to pamaquin (nausea, vomiting, abdominal pains, precordial pain, etc,). Acute haemolytic anæmia may occur. Isopentaquin a new drug which is said to be more effective and less toxic; it is still in experimental stage.

(*d*) *Camoquiin*:—CAM-AQI now called Camoquiin (P.D.) is a light yellow crystalline powder soluble in water. It is a quinoline derivative, the chemical formula being 4 (3′-diethalminomethyl-4′-hydroxyanilino)-7-chloroquinoline dihydrochloride dihydrate. The absorption, distribution and excretion of the drug is similar to that of quinacrine but it causes little or no staining of the tissues. Each tablet contains 0.05 gm. of the base. Chaudhuri and Chakraverty (1948) treated 54 malaria cases using 2 dosage schedules, the first being 0.1 gm. twice daily for 3 days and the second, a single dose of 0.5 gm. (about 10 mg. per kilo body weight). The therapeutic response was very quick in all

series. The temperature came down to normal by the second day of treatment in 85 per cent of the cases and by the third day in 98 per cent. The peripheral blood was also rapidly cleared of asexnal parasites which could not be seen beyond the second in 83 per cent and beyond the third day in 100 per cent. The drug had little or no effect on gametocytes, and relapses were less with the single dose. No untoward symptom was encountered. In view of the satisfactory response with a single dose, Camoquiin may prove a suitable remedy for mass treatment in rural areas. *Efficacy of atebrine.*

(ii) Atebrin (Mepacrine)

Atebrin:—The work of Schulemann and his colleagues which culminated in the synthesis of plasmoquin, was continued. More than 1,200 compounds were prepared and eventually the drug originally called 'erion' and now known as atebrin was produced. Chemically it is the dihydrochloride of an alkylamine-acridine derivative and is a yellow powder with a bitter taste. Its solubility in water is 1 in 14 and it forms a neutral fluorescent solution. The action of atebrin is very similar to quinine, but according to some workers it is more effective than quinine in reducing the number of relapses. None of these drugs by itself nor any combination thereof, such as atebrin and plasmoquin or quino-plasmochin, represents a *therapia magna sterilisans*.

Chopra and his co-workers (1933) carefully tested this drug on the Indian strains of malaria and have concluded:—

(1) Atebrin is an effective drug in the treatment of Indian strains of malaria. Its destructive action on the asexual forms of benign tertian, malignant tertian and quartan types of malaria is about equal, the schizonts disappearing from the peripheral circulation after 0.6 to 0.9 gm of the drug.

(2) The sexual forms or gametocytes are more slowly acted upon than the asexual forms. The gametocytes of the benign tertian and quartan types are readily destroyed. The gametocytes of the malignant tertian type are not affected at all.

(3) The drug is effective in doses of 0.1 gm three times a day, the course lasting for five days, making a total of 1.5 gm of the drug for the cure, Recently a 7 to 10 days course is recommended in severe cases. The drug can be given intravenously in doses of 0.1 gm to 0.3 gm dissolved in 3 to 9 ccm of distilled water respectively when the number of parasites in the peripheral blood is large.

Atebrin musonate is the soluble form of atebrin and was largely used in the epidemic in Ceylon. It is an atebrin salt suitable for parenteral route, 0.3 gm of atebrin being contained in 0.375 of the salt. Hecht has shown that when atebrin is administered by mouth much of it is retained in the upper intestine, liver and bile, and only the overflow goes to the peripheral circulation after these organs are saturated. This exceptionally high concentration of atebrin is responsible for the manifestation of toxic symptoms. By injections, the drug gets into the circulation quicker and gives response earlier. Doses from 0.1 to 0.375 gm are given and a single injection often produces remarkable effects on the clinical symptoms, but recrudescence usually occurs in a few days. Two similar injections on successive days, proved sufficient to control the temperature within 48 hours, and in four days in all forms of benign tertian. *Soluble atebrine.*

Both quinine and atebrin have been combined with plasmoquin in preventing relapses and for prophylaxis. *Combinations.*

Atebrin and Plasmochin combinations—As atebrin only acts on the asexual forms of malignant tertian parasites it has been combined with plasmoquin. A comparative study of the action of atebrin and atebrin-plasmochin combination on Indian strains of malaria by Chopra and co-workers (1936) showed that, (1) in cases of benign tertian and quartan malaria the combination of the two drugs is not more effective than atebrin alone in so far as the time of disappearance of the parasites from the blood is concerned; (2) in the case of malignant tertian infection, however, the combination appears to be more effective and the parasites disappear more rapidly from the peripheral circulation: (3) with regard to the relationship between the number of parasites and their disappearance from the peripheral circulation, atebrin alone and atebrin-plasmochin combination behave in the same way; (4) the relapse rate is lower in cases when the combination of the two drugs is used; (5) the combination of the two drugs is more toxic.

It is therefore recommended that in those cases where crescents are present, a 3 days course of plasmoquin 0.01 gm twice daily be given after the course of atebrin is completed. The toxic effects are thus minimised. Combination of atebrin and quinine have also been used. Much work was done concerning the effectiveness and toxicity of mepacrine during the World War II. Mepacrine is not uniformly distributed in blood and tissues but is rapidly taken up by the body cells. The concentration in blood according to Hamilton Fairely (1945) is 2.2 micrograms per 100 ccm, but it is likely that the drug absorbed by the cells also exerts antimalarial action. In the army during the war doses of 0.1 gm twice daly for two days in the week were used with good results, but 0.1 gm daily suppressed an attack under all conditions (physical exertion, cold, altitude etc.) in volunteers artificially infected by mosquito bites. If this dose was taken for 4 days in a week, a few developed malaria. To prevent the development of malignant tertian malaria, it was necessary to give 0.1 gm daily for 28 days after the last infection.

Prolonged use of mepacrine for periods as long as 18 months produced no toxic effects.

Curative action:—During World War II, in cases of malaria with presence of parasites, fever, anæmia, enlarged spleen etc. 5 grains of quinine were given in doses of from 0.1 to 0.3 gm three times daily for three days. The advantage of giving quinine at first is that it acts quickly and gives the patient relief from symptoms.

(iii) Sulphonamide compounds

In view of the large number of bacterial infections which have been successfully treated with sulphonamide compounds, it is not surprising that these drugs should have been tested in malarial infections. The first observations were made by Diaz de Leon (1937), who claimed to have successfully treated several cases with rubiazol. These results were confirmed by Chopra and Das Gupta (1938) and others who showed soluseptasine, sulphapyridine, sulphathiazole, sulphadiazine have a specific action on *P. Knowlesi* infections in monkeys and plasmodial infections in man. These compounds are not suitable for treatment of malaria.

(iv) Paludrine (No. 4888)

The workers of the Imperial Chemical Industries Laboratories started work in connection with the antimalarial drugs from an entirely new aspect. It has never been confirmed that injected sporozoites penetrate the red blood corpuscles directly. There is always a period of days following the bite of a mosquitoe

before the parasites be demonstrated in the blood. Until recently the immediate fate of the sporozoites was uncertain, but it has now been shown that a period of development of the parasite occurs in the solid tissues and cells of the reticulo-endothelial cells of the vertebrate host, before the red cells are invaded; it is the duration of this tissue phase which constitutes the incubation period. The development phase occuring during this period is the post-sporozoite phase or the tissue phase (primary exo-erythrocytic phase) and the parasites at this time are called *cryptozoites*, or extra-erythrocytic forms.

After this period of incubation the parasites come out of the endothelial cells and begin their erythrocytic phase. They enter into the red blood corpuscles within which a generation of parasite is formed leading from the trophozoite form to the segmented forms. The corpuscles containing the segmented forms (Schizonts) of parasites rupture ushering in the clinical picture and setting free the new merozoites to begin the next phase, It is possible that these merozoites are not all killed or partially destroyed. In cases of relapse possibly the following cycle takes place:—

The young merozoite on entering the endothelial cells continues its development as the secondary exo-erythrocytic phase. Parasites inside the endothelial cells of primary exo-erythrocytic phase also continue their development into the stage of secondary exo-erythrocytic phase. After a course of time, the parasites undergo certain changes in forms and are liberated in the circulation to enter the red blood corpuscles where they go through the erythrocytic phase of development. These erythrocytes rupture, liberate the parasites and produce the clinical picture of relapse.

The conception of primary exo-erythrocytic phase explains the enigma of incubation and of secondary exo-erythrocytic phase that of relapse and recrudescence.

The exo-erythrocytic forms are known to occur in almost every type of avian malaria and are found in endothelial cells in the tissues. They occur as a stage between the sporozoites introduction by the infected mosquito, and the parasites which eventually get within the erythrocytes; they may also persist through the time an infection lasts, and serve as a reservoir from which parasites may be released to invade more blood corpuscles.

Short and Garnham discovered the continued existence in monkey malaria of the exo-erythrocytic forms in the liver after establishment of blood infection and after the parasites disappeared from the peripheral blood. They suggest that this persistance of the exo-erythrocytic state plays an essential part in the maintenance of the infection over long periods and in the production of relapse.

That an exo-erythrocytic form must occur in association with human malarial infections seemed certain both from attempts at transmission of human malaria, from chemotherapeutic experiments, and from the fact that when human beings are infected both with Plasmodium falciparum and P. vivax the growth of P. falciparum successfully interferes with the further development of P. vivax, which finds difficulty in appearing in the blood so long as the body is infected with *P. faiciparum,* an instance of interference, a phenomenon well known in connection with viruses.

Subsequently Shortt, Farnham, Covell and Shute (B. M. J. March 20, 1948) have shown presence of pre-erythrocytic stage of human malaria, *Plasmodium vivax*.

In view of this there are three possible targets in the chemotherapy of malaria, namely the sporozoites, the tissue phase and the blood phase. Clinical symptoms do not appear till the blood phase and therefore all efforts till recently were naturally directed towards this phase. From this point of view quinine and atebrine were great specific drugs in the treatment of malaria. In all experimental work therefore the blood phase was used and it culminated in Roehls test. The search of a drug which would act on the sporozoites or the tissue phase preventing the parasite from appearing in the blood was not attempted. For this purpose laboratory infection in chicks with *P. gallinaceum* was established and a technique was evolved by which action on the sporozoite or the tissue phase could be tested. Investigations were started with anilinopyromidine derivative and thousands of compounds were synthesized and tested. To compound No. 4888 the name of Paludrine was given. Administered orally it is absorbed rapidly and almost completely; the peak levels are reached within three hours of administration. Fall away in concentration is rapid, although sufficient may remain after twelve hours to cause a build-up if the drug is taken twice daily; this build-up reaches its maxmum after four to six days (Maegraith, Adams et al. 1945). The concentration of 'Paludrine' in the red blood corpuscles is about four times that in the blood plasma.

Clearance.

Excretion is more rapid than mepacrine. Even after a dosage of 0.5 gm. twice daily, the blood concentration falls below assayable level within one week from cessation of the dosage and the drug cannot be detected in the urine after nine days. Because of rapid excretion no ill effects have been reported following long continued use as a prophylactic.

Toxicity.

In animal experiments, it has no adverse effects on blood pressure, respiration or intestinal movement.

In man, even minor toxic side-effects have not been observed except after the administration of comparatively large doses. In a proportion of cases receiving 0.5 gm. twice daily, vomiting and epigastric discomfort have been reported. In most instances the symptoms could be prevented if the drug were taken with a glass of water, and they were usually only apparent during the first few days of the treatment. Even when the treatment was continued for as long as 28 days and when the dose was increased to 0.75 gm., given twice daily for a similar period, no further toxic by-effects were manifested (Maegraith, Adams et al, 1945). A single dose of 1 gramme produced diarrhoea and haematuria, but there was no evidence of parenchymal damage to the kidney.

Curative.—In the treatment of malaria two courses of action may be adopted, either (*a*) clinical cure by which is meant reduction of the temperature to normal, and control of the acute attack; or (*b*) radical cure, that is to say, complete eradication of infection.

Malignant tertian, Benign tertian, and Quartan malaria.

Fairley et al (1946) have shown that a single dose is sufficient to end in attack of malaria and bring the temperature to normal. Their results have been amplified and extended by trials in Britain and India.

It is desirable that for the clinical cure of all types of malaria a "single dose" of 0.3 gm. (three tablets) should be given daily till symptoms are controlled.

To obtain a radical cure a dose of 0.1 gm. (one tablet) three times daily for ten days should be given. *Malignant tertian malaria.*

In a short course of treatment it will not radically cure all cases of benign tertian malaria. Some will relapse although the periods between relapses are longer after its use. *Benign tertian malaria.*

It has been shown, however, that a dose of 0.1 gm to 0.3 gm twice weekly for six months at spaced intervals, after the acute attack has been controlled, will hold off relapses indefinitely. There are theoretical reasons for supposing that by this continued suppression, the persistent forms of the parasite in vivax malaria will be eradicated.

Because of its ease of absorption and rapid effect when given by the mouth, Paludrine has not been used to any great extent by the parenteral routes. Doses of 0.1 gm., however, have been given intravenously without demonstrable toxic effects and have been used successfully in the relief of cerebral malaria. *Parenteral administration.*

Malarial Protection

Causal Prophylaxis.—By causal prophylaxis is meant killing the parasite before it enters the red blood cells. To achieve it a drug must kill either the sporozoites or the exo-erythrocytic forms. No drug is known which will kill the sporozoites, and 'Paludrine' in easily tolerated doses, will act as a complete causal prophylactic in malignant tertian malaria. 0.1 gm. (one tablet) taken at approximately half-weekly intervals, *e.g.* Wednesdays and Sundays, would give complete protection against malignant tertian malaria.

It has also a partial causal prophylactic action against benign tertian malaria, but dosage which might achieve complete causal prophylaxis would probably be impracticable.

Clinical Prophylaxis.—By clinical prophylaxis is meant inhibition of parasite development to such a degree that malarial symptoms do not appear. To achieve this effect paludrine 0.1 gm. once a week will usually prove satisfactory in many areas where malaria is endemic; but where it is hyperendemic it will be necessary to increase the once-weekly dose to 0.3 gm. or three tablets.

A once-weekly dose of 0.3 gm. is only recommended when the administration of 0.1 gm. twice weekly (at three or four day intervals) is inconvenient. The twice-weekly treatment should be followed wherever possible because, in addition to suppressing benign tertian malaria, it will give complete causal prophylaxis against malignant tertian malaria.

Gametocidal Effect.—The drug is not gametocidal in man in the accepted meaning of the term, but it prevents the development of the gametocyte in the gut of the vector mosquito. This effect depends on the female mosquito imbibing a small quantity of the drug, along with the gametocytes from the host's blood. Astonishingly small concentrations seem to be effective, because a patient who has been receiving 0.1 gm. of the drug thrice daily, is still incapable of transmitting infection about a week later, although by this time the concentration of the drug in his blood is too low to be estimated by the available biochemical methods.

(3) Cinchona Alkaloids Versus Synthetic Anti-Malarial Drugs

What is going to be the effect of the introduction of these powerful synthetic antimalarial drugs on the cinchona alkaloids? Are these old veterans going to be entirely replaced by the new-comers? The Malaria Commission of the League of Nations pointed out that neither of the two groups, however intensive the treatment may be, is *therapia magna sterilisans* and their effect in preventing relapses is not marked. They also have no true causal prophylactic action in all forms of malaria. The synthetic drugs, according to the Commission, are not to be regarded as 'substitutes for quinine but as additional weapons for use in particular circumstances and for special purposes'.

Quinine is an effective drug against malaria and its combination with mepacrine, pamaquin and pentaquin is definitely advantageous. Its sedative action on the central nervous system and heart is further a great asset in controlling certain symptoms of malaria such as headache, malaise, general pains, etc.

This is probably the correct point of view. The cinchona alkaloids have a very low degree of toxicity and can be used with impunity in the mass treatment of malaria and even self-medication by the patient is without any danger or serious harm. The same cannot be said about the synthetic antimalarial drugs; it yet remains to be seen whether paludrine will satisfy this criterion. Generally they have a high degree of efficiency combined with toxicity and should be used under proper medical supervision. Even under such conditions,toxic effects are met with. Unless paludrine has a low enough toxicity, the cinchona alkaloid will hold the field in the treatment of malaria among the masses generally, in this country.

During the last few years, it has been debated whether the cinchona plantations in this country should be extended. One of the arguments brought forward against their extension is that in view of the rapid development of synthetic antimalarial drugs there will be no further necessity for cinchona alkaloids. The writer has no hesitation in saying that unless paludrine is proved to have all qualities desired for the next 10 to 15 years, we shall have to use the cinchona alkaloids in our struggle against malaria in India. The cinchona plantations take 7 to 8 years to mature and if we wish to extend the treatment of malaria among the masses, which is one of the chief methods of eliminating this disease, it is very desirable that cinchona plantations should be extended till such time as an effective, cheap and equally harmless synthetic drug is available which can be used for mass treatment of malaria without any special supervision.

In India large scale and economic production should be taken in hand now that information is available regarding the species of cinchona which yield the highest percentage of the alkaloids and the best soil for cultivation.

PART III

CHAPTER V

SPIRAL DISEASES

SYPHILIS: PATHOLOGY AND CLINICAL ASPECTS: ACQUIRED SYPHILIS, NEURO-SYPHILIS, CONGENITAL SYPHILIS; TREATMENT OF ACQUIRED SYPHILIS: CHANCES OF CURE, TREATMENT OF EARLY SYPHILIS, MODERN INTENSIVE THERAPY, PENICILLIN IN TREATMENT OF SYPHILIS, THERAPY OF LATE SYPHILIS—YAWS—THE RELAPSING FEVERS—RATBITE FEVER—OTHER SPIROCHAETAL DISEASES: INFECTIOUS JAUNDICE (WIEL'S DISEASE), JAPANESE SEVEN DAYS FEVER, (NANUKAYAMI)—ARSENICAL COMPOUNDS: PHARMACOLOGICAL ACTION; ARSENIC COMPOUNDS; MODES OF ADMINISTRATION; ORGANIC COMPOUNDS OF ARSENIC: ATOXYL, TRYPARSAMIDE, STOVARSAL (ACETARSONE), TREPARSOL, ARSPHENAMINE, NEO-ARSPHENAMINE, OXOPHENARSINE HYDROCHLORIDE (MAPHARSIDE), SULPHARSPHENAMINE, THIOSARMINE; TOXIC EFFECTS OF ARSENIC COMPOUNDS: IMMEDIATE, EARLY AND LATE REACTIONS, TREATMENT—BISMUTH AND ITS DERIVATIVES: PHARMACOLOGICAL ACTION; THERAPEUTIC USES; MODES OF ADMINISTRATION; TOXIC EFFECTS, TREATMENT; BISMUTH COMPOUNDS; SUMMARY—MERCURY AND ITS DERIVATIVES: PHARMACOLOGICAL ACTION; THERAPEUTIC USES: MERCURY IN SYPHILIS, PROPHYLACTIC USES; MODES OF ADMINISTRATION; TOXIC EFFECTS; PREPARATIONS—IODIDES IN SYPHILIS.

DISEASES CAUSED BY SPIRAL ORGANISMS

Spirochaetes are organisms which have flexible bodies. These organisms were placed by Schaudinn among the protozoa while other workers have refuted this and have placed them as being more closely related to bacteria—class *Schizomycetes,* order *Spirochaetales.* Dobell considers that they belong to a special group of the *Protista* which he calls *Spirochaetoides* as they have characteristics common to both bacteria and protozoa. Spirochaetes have no definite nucleus (as have protozoa); they reproduce by transverse binary fission into two equal forms, and have an anterior and posterior end. There are no flagella but they have some inherent sinuous and rotating movements and power of motility. The pathogenic spirochaetes are more flexible than bacteria. They do not form spores, but granular forms have been observed and described as representing a stage in the life cycle of the organism; filtrates containing such granules have been shown to be infective, but it is possible that some flexible spiral forms may go through bacterial filters. Infectivity of granular forms is, therefore, still not proved. Spirochaetes in the animal body are susceptible to the destructive action of compounds of antimony, arsenic, bismuth and mercury.

Classification.

Attempts to subdivide the members of this group have not been very successful. The common spirochaetes of man and animals are given in the following table (Medical Protozoology, Das Gupta):—

Treponema	*recurrentis*	Man	Blood	Relapsing fever.
„	*pallidum*	„	Tissues	Syphilis.
„	*pertenue*	„	„	Yaws.
„	*carateum*	„	Skin	Pinta.
„	*vincenti*	„	Skin	? *Ulcus tropicum* (Naga sore).
			Throat	? Vincent's angina.
„	*dentium*	„	Mouth	—
„	*medium*	„	„	—
„	*buccalis*	„	„	—

Treponema	*macrodentium*	Man	Mouth	—
„	*mucosum*	„	„	—
„	*eurygyrata*	„	Intestine	? Spirochaetal dysentery
„	*stenogyrata*	„	„	—
„	*intestinalis*	„	„	—
„	*bronchiale*	„	Bronchi	Bronchitis.
„	*refringens*	„	Genitalia	—
„	*gracile*	„	„	—
„	*theileri*	Cattle	Blood	Relapsing fever.
„	*equi*	Horses	„	„
„	*ovinum*	Sheep	„	„
„	*suis*	Pigs	„	„
„	*cuniculi*	Rabbits	Tissues	Rabbit syphilis.
„	*anserinum*	Geese	Blood	Relapsing fever.
		Fowls	„	„
		Ducks	„	„
Leptospira icterohaemorrhagica		Man	Blood	Weil's disease (Acute infective jaundice).
Leptospira hebadomadis		„	„	Japanese seven-day fever.
„	*canicola*	Dog	„	Stuttgart dog plague.
		Man	„	Canicola fever.
„	*grippotyphosa*	Man	„	Swamp fever.
Spirillum minus		„	„	Rat-bite fever (Soduku).

T. recurreutis and *T. duttoni* which produce relapsing fever are now placed under genus *Borrelia*.

The pathogenic spiral organisms of man may be classified as follows:—

1. *The blood-inhabiting spirochætes, viz.,* those of the relapsing fevers, and *Spirillum minus,* the parasite of rat-bite fever.

2. The *Leptospira group* including the parasites of Weil's disease, and Japanese seven-day fever.

3. The *Treponema group* including the spirochætes of syphilis and yaws.

4. *Vincent's spirochæte,* which together with the fusiform bacillus is responsible for Vincent's infection.

The Relapsing fever spirochœtes. The spirochæte of relapsing fever was first described by Obermeier (1873) and was named *Spirochæta obermeieri.* Since then a large number of different species of the relapsing fever spirochætes, *e.g., T. recurrentis, T. duttoni, T. carteri,* have been described by workers in different parts of the world. It is very doubtful, however, whether these so-called species represent anything more than local races of one and the same spirochæte. Many workers have shown that it is impossible to distinguish the various strains from their morphological characteristics. It is better, therefore, to conclude that there is but one species of relapsing fever spirochæte, *Treponema recurrentis.* The organism, when observed alive under dark ground illumination, shows the axis of its spiral nearly always straight and its coils uniform. When an infection is abating as the result of the development of antibodies in the blood, various coiled and clumped organisms may be found In stained films, the spirochætes show a very irregular appearance with widely open coils, twists and every possible variation in type.

T. recurrentis passes through a Berkfeld filter and infected blood, diluted with ten parts of saline-citrate solution under fifty pounds pressure, is infective to rats. The filterability is attributed by some to a granule phase, while others have found definite spirochaetes in the filtrate. T. pallidum is said not to pass through this filter.

Spirillum minus, the cause of rat-bite fever, is commonly met with in India. This organism is found in the blood of infected mice, rats or guinea-pigs during the first two weeks and then becomes distributed in the connective tissues of the lips, nose, and tongue. It has been found that about 3 per cent. of house rats are carriers of the disease.

The Leptospira group. These organisms are characterised by the fact that the body consists of a spirally wound thread, the spirals being so fine that unless careful inspection is made they are overlooked. Under the dark-ground, the leptospira look like brilliantly refractile swimming pieces of rope. The two diseases due to leptospira infection in man are:—(1) Weil's disease due to *L. icterohœmorrhagica,* (2) Japanese seven-day fever due to *L. hebedomadis.*

Treponema pallidum. The spirochæte of syphilis has essentially the same structure as the relapsing fever spirochætes, from which it differs only in being smaller and in having a larger number of coils for a given length. These are essentially parasites of the tissues and of the lymphatic system and include the following species:—(a) *Treponema pallidum,* the spirochæte of syphilis, (b) *Treponema pertenue,* the spirochæte of yaws, and (c) *Treponema cuniculi,* a spirochæte of the rabbit.

The discovery of the spirochæticidal power of arsenic and its derivatives marked the dawn of a new era in chemotherapy. The researches of Ehrlich and many other renowned workers that followed him were directed primarily to fight the organism of syphilis, *i.e., T.pallida.* The success attained in this direction has done much towards solving the problem of spirochætal infections in its protean manifestations.

1. Syphilis

Although syphilis was not a very serious problem in India in the past, the two World Wars have increased both the incidence and the severity of this disease in this country. It has therefore been described briefly below:—

(1) Pathology and Clinical Aspects

Historical.

That syphilis, which formerly was a dreaded disease, which often led to the most tragic results in spite of treatment extending over years, can now be effectively controlled in all its different phases, is one of the greatest achievements of medical science. The causative micro-organism was discovered by Schaudinn and Hoffmann in 1905, and in 1906 Wassermann developed serum diagnosis by the complement fixation test. The classical work of Ehrlich led to the producing of an effective arsenical compound in 1909, and in 1921 the value of bismuth compounds in the disease was demonstrated.

Syphilis usually runs its course in a number of well-defined stages. The primary stage starts with the appearance of a characteristic sore at the site of inoculation, and is followed a few weeks later, by the secondary stage with its widespread lesions, principally in the skin and mucous membranes. In the tertiary stage, the lesions, usually few in number, are individually large and more destructive than in the secondary stage, and appear after a latent period lasting from a few months to many years after the subsidence of the secondary stage. Tabes dorsalis and general paresis (G.P.I.) are now generally regarded as manifestations of quarternary syphilis.

Mode of infection.

Infection occurs through the entry of the *Spirochaeta pallida,* present in the discharges, e.*g.*, semen, saliva, or in the blood, either through a breach in the skin or mucous membranes, or directly into the blood stream. The organisms are present on the discharges in the early part of disease and transmit infection to others. Experiments on rabbits show that the blood is infective from a week or earlier after inoculation, and in syphilis, the blood is infectious for animals, chiefly in the primary and secondary stages, but very rarely in the tertiary stage. That the infectivity of the blood usually dies out quickly after the first year is shown both by experimental and clinical evidence. Repeated transfusion to non-syphilitic patients, from cases suffering from late manifestations of syphilis, does not produce infection in the recipients. The semen has been shown to be infective very much longer.

The commonest method of infection is through sexual intercourse, and it has been estimated that only 8 or 9 per cent of the infections are extra-genital. The majority of extra-genital chancres are found on the tongue and lips, and next in frequency come the breast and nipple. Syphilitic tissue excised 96 hours previously are infective to animals, but discharges become non-virulent in a few hours. Post-mortem material 48 hours after, has produced infection. *T. pallidum* is destroyed by drying and weak antiseptic solutions. Low degress of heat (50°C) kill it. *T pallidum* can bore its way rapidly through the cells of the body and this explain its power of reaching all parts of the body.

Causal organism.

The *Spirochaeta pailida,* also known as *Spironema pallidum* or *Treponema pallidum,* appears as a motiie, deadwhite, delicate, corkscrew shaped organisms with clear cut very regular coils. Its length varies from 4 to 14; its thickness is 0.25 g; the distance from crest to crest of any two coils is 1 to 1.24 (7 coils to the width of a red blood corpuscle.) In dried films, it is very easily stained by silver nitrate. It is best shown in discharges from syphilitic lesions examined under dark ground illumination. *S. pallida* is indistinguishable morphologicaily from *S. pertenues* of yaws.

Recently the unitarian theory of disease due to the spirochaete, Treponema, has been propounded. It is believed that there is only one species, T. pallidum, propagated both by venereal and non-venereal routes. The forms of disease caused by this organism are characterized by early and late stages, separated by a latent period, with characteristic pathological response to treatment with heavy metals and penicillin. The name "*Treponematosis*" is suggested for these conditions which includes all forms of yaws, framboesia, syphilis, etc. *Treponema* has been adopted as the generic name for the microscopic parasite.

Pathology. Primary ulcer.

It is doubtful whether *S. pallida* can penetrate the unbroken skin or mucous membrane; it usually enters through an invisible breach of the surface. It rapidly penetrates deeper and reaction occurs in the subepithelial tissues where collections of lymphocytes and plasma cells occur around the lymph capilliaries and small blood vessels. This infiltration becomes marked, the covering epithelium is thickened and œdematous, and finally ulceration occurs. The lymphatic glands draining the area, undergo chronic inflamatory changes.

Secondary ulcer.

In the secondary stage, the skin lesions are usually macular or papular, and there may be increase or decrease of pigmentation in the affected areas. Lesions of the mucosa of the mouth, fauces and anal canal, are similar to those in the skin.

Tertiary lesions.

Tertiary skin lesions consist of granulomas composed of collections of epithellial cells, plasma cells and numerous lymphocytes. The centre of the lesion becomes necrosed, and ulceration results. In the subcutaneous and submucous tissues, muscles, bones and testes, tertiary lesions are discrete or diffuse, and there is tendency to degeneration and eventual fibrosis.

Milder course in females and in tropics.

It is well known that syphilis is generally a milder disease in females. Spirochaetes, though common in the testes of old syphilitics, are very rare in the ovaries. It has been suggested that possibly the female sex hormones are responsible for this. In warm climates syphilis is also a comparatively milder disease. Cardiovascular and quarternary nervous manifestations are not so frequently met with in India as in Western countries.

Serum tests.

The most valuable test for early syphilis is the microscopic examination of the juice of the lesion, containing as little blood as possible, for *S. pallida* under the dark ground illumination. Serological tests are divided into the complement fixation or Wassermann test, and the flocculation or precipitin tests. In addition to these tests, the cerebrospinal fluid is usually tested for increase of globulin, increase of cells (above 5 cells per cmm being generally considered pathological), and for one of the colloidal reactions, *e.g.,* gold, benzoin or mastic.

False positive reactions.

It is generally agreed by most authorities in India, that a very large number of false positive Kahn and Wasserman reactions are met with in Indian patients hospitalized for a variety of tropical diseases. Such false positive reactions frequently become negative without any anti-syphilitic treatment, and are most frequently encountered in malaria, kala-azar, leprosy, yaws, trypanosomiasis, pinta, and relapsing fever.

(i) Acquired syphilis

Primary stage.

1. *Primary stage*:—The incubation period varies from 10 to 90 days or longer, the most common being from 3 to 4 weeks. The longer incubation periods may be due to unsuccessful attempts at prophylaxis by local disinfection or to the taking of antisyphilitic drugs by mouth or by the parenteral route. The primary sore, known as a chancre, then appears. Chancres are generally single but may be multiple. The commonest site in men

is the coronal sulcus, other sites being the prepuce, frenum, urinary meatus, or dorsum of the penis. In the female, the primary sore is often found in hidden situations *e.g.*, on the labia, clitoris, mouth of urethra, posterior commissure, remains of the hymen and the os uteri. It gives rise to little or no discomfort.

Diagnosis.

The blood serum gives a positive Wassermann and positive flocculation tests in more than 50 per cent of cases, 10 to 14 days after the appearance of the primary sore. About 4 to 6 weeks later, almost 100 per cent of cases are positive. The cerebrospinal fluid may sometime show changes even before the serum is positive.

Certainty and speed of diagnosis of primary syphilis are of paramount importance, as the outlook is best when treatment is started before serum-reactions become positive. Diagnosis of primary syphilis should never be made unless *S. pallida* are found and the serum test is positive. The serum tests should be made as a routine on all patients at first appearance. The most characteristic distinguishing sign of primary syphilitic sore is the rubbery induration of the edge and surrounding tissue, and the comparative painlessness of the lesion. Marked enlargement of the inguinal glands without pain or evidence of active inflammation is often a very valuable sign.

The commonest non-syphilitic lesions on the genital area from which syphilitic chancres must be distinguished are chancroid or soft chancre, herpes vesicles, balanitis, granuloma venereum, the primary lesion of lyphogranuloma inguinale, gonorrheal sores, and malignant disease.

Secondary stage.

2. *Secondary stage*:—This stage is one of generalization of external lesions, and is characterised by outbreaks of rashes of various kinds all over the skin, and by lesions of the mouth, throat and anal canal. The first outbreak generally starts from 8 to 12 weeks after infection, the most constant pre-erruptive sign being moderate enlargement of lymphatic glands throughout the body. The glands are not tender, and are readily felt in the groin, axilla, epitrochlear region, and the posterior triangles of the neck. Mild fever of a remittent type, may be present, usually lasting for a few days, but it may persist for several weeks. Symptoms indicative of syphilitic activity of the meninges, such as headache, increased reflexes, slight paraesthesias, aches and pains in the limbs, etc. are encountered.

Skin lesions.

The first rash may appear overnight, distributed all over the trunk and limbs, or it may be confined to one part from which it may or may not spread to the limbs or head. The parts subject to irritation are often more affected. The macular or reseolar syphilide which is usually the first to appear consists of round or oval well defined spots varying in size from a pin-head to about a eight-anna piece. The spots are at first light red, later become darkened, and may end as brown stains. If no treatment is given, the roseola fades after some weeks, but it may reappear with fever leaving large areas of normal skin between. These individual lesions are annular, circles or half circles.

The lenticulo-papular eruption usually follows the roseola, but it may be the first secondary eruption. It appears as dome-shaped papules, which vary in size from a lentil to a pea. *S. pallida* can almost always be found in the serum which oozes from the papules. Degeneration of the papules may result in scaling and the rash may then be mistaken for psoriasis. In neglected and debilitated patients the papules may become pustular, and the whole body may be covered with crusts. A more severe degree results in syphilitic rupia. Loss of hair (syphilitic alopecia) occurs in a large proportion of cases of secondary syphilis.

Mucous membrane

Secondary lesions of the mucous membrane are essentially papular, the centre of the lesion is occupied by a thin white pellicle, and round this is a raised red sharply defined velvety border. These mucous patches extend and become confluent, producing the classical snail-track ulcers. The commonest sites for such patches and ulcers are on the mucous membranes of the lips, cheeks, pillars of the fauces; the tonsils, uvula, and the soft palate may be affected suggesting diphtheria or Vincent's angina. The labia, vagina, cervix, prepuce or glans, and the anal canal may be affected. These mucous patches are generally full of spirochaetes.

Gummata.

3. *Tertiary stage*:—Lesions of the skin occur as (1) tubero-serpiginous syphilides (a central nodule surrounded by a ring of similar nodules) with a tendency to degeneration and scaling and eventual cicatrization, or in formation of small ulcers. (2) The single gummatous ulcer originates in the subcutaneous tissues, muscle, or bone, as round elastic painless swellings which merge with the skin, breaking down in the centre; they give rise to the classical tertiary ulcers, with base covered with a washleather slough and punched-out indurated edge.

Myositis.

A diffuse interstitial myositis may affect any muscle, particularly one of the flexors; individual gummata may form in one or more muscles, and may break through to the skin, or retrogress and lead to shrinkage of the whole muscle, which may calcify or even ossify.

Periostitis.

Localised gummatous periostitis may appear on any bone as a smooth swelling connected with the underlying bone, but the clavicles, ribs, sternum, crenium and tibias are commonly affected. The swelling may undergo ossification and remain as an ivory hard boss on the bone, which is of diagnostic value. The centre of the swelling may break and result in a funnel-shaped gummatous ulcer, with a bare bone base. A diffuse periosititis is far commoner in congenital syphilis producing sabre-scabbard tibia.

Nasal lesions.

Gummatous infiltration of the nasal bones leading to necrosis and offensive discharge is important, and unless promptly treated, may result in serious deformity. Eventually the bridge of the nose may be destroyed and fall in, and the palate may be perforated.

Arthritis.

Gummatous arthritis which is painless, most commonly involves the knee, which is uniformly enlarged; there is little or no muscular wasting round the joint. Gummatous nodules may be felt in the synovial membrane.

Charcots joints may occur in tabes, and are diagnostic of the pre-ataxic stage. The joint attracts attention as it suddenly becomes distended with fluid, without pain or inflammation, or may suddenly give way or become dislocated without apparent cause.

Testicular lesions.

Tertiary lesions of the testis are either a diffuse interstitial orchitis or localized gumma, but the two types are often combined. The testis is at first evenly enlarged, hard, elastic, and heavy; the normal testicular sensation is lost. Gummata are usually multiple and seen as smooth, hard, easily palpated bosses, which may break down and ulcerate. Hydrocoele commonly occurs and early tapping may become necessary. The epididymis is not usually involved.

Leucoplakia.

Leucoplakia of the tongue and cheek is common in the later stages of syphilis. Interstitial glossitis occurs, the tongue becomes smooth, red and glazed. Gumma of a tonsil may appear.

Cardio-vascular lesions.

In western countries, syphilis is responsible for 10 to 15 per cent of all cardiovascular disease, and this type is said to be responsible for about 30 per cent of all deaths from syphilis. The incidence is very much lower in females than in males. In India, however, cardiovascular lesions are rarely encountered, even in the comparatively temperate regions of the Punjab and Kashmir.

The commonest lesion is situated in the ascending arch of the aorta, and may result in narrowing of the mouths of the coronary arteries, or in the production of aneurysm. Discrete gumma of the myocardium is very rare, and may produce various types of heart-block. The aortic lesion is usually symptomless at first. Later, symptoms due to the development of aortic incompetence may develop. Anginal attacks may occur due to involvement of the coronaries, and attacks of coronary thrombosis are not uncommon. When aneurysm of the aorta develops, there may be severe boring pain in the chest, due to erosion of bone. Sudden death may occur as a result of rupture of an aneurysm.

(ii) Neuro-syphilis

Syphilis is one of the common causes of organic disease of the central nervous system. The interval between the primary infection and involvement of the central nervous system may vary from one month to forty years. Meninges and vessels may be involved early but the lesions in which there is involvement of the nervous parenchyma such as general paralysis of the insane or Tabes dorsalis do not occur earlier than 14 to 15 years after the infection. If well marked skin reaction develops neurosyphilis may not develop and vice versa. This may be due to the type of the spirochaete having specific predilection. Neurosyphilis is much commoner among males than females. The disease may be limited to the intracranial structures or spinal structures and may involve mainly either the nervous parenchyma or the meninges and blood vessels.

Harrison has classified neurosyphilis into:—

Classification of neurosyphilis.

1. *Intracranial syphilis*
   - (*a*) The brain substance is affected, gummata form and general paralysis of the insane is the result.
   - (*b*) The meninges are affected producing cerebral meningitis.
   - (*c*) The blood vessels are affected producing cerebral vascular syphilis.

2. *Spinal Neurosyphilis*

(*a*) The spinal cord tissue is affected producing tabes dorsalis, progressive muscular atrophy, Erb's syphilitic spastic paraplegia.

(*b*) The spinal meninges and interstitial elements of spinal cord are affected, gummata form, producing acute syphilitic transverse myelitis, subacute and chronic syphilitic meningo-myelitis.

(*c*) When blood vessels are affected, spinal vascular syphilis occurs.

Neurosyphilis may occur in congenital syphilis resulting in mental deficiency, infantile hemiplegia, juvenile tabes dorsalis.

General paralysis of the insane

General paralysis.

The disease usually begins between 8 to 20 years after the infection and is seen in 10 per cent cases of neurosyphilis in Western countries. It may occur in adolescence as a result of congenital syphilis.

Mental symptoms.

Mental changes which may take the form of either dementia, or delusions of grandeur type, are the first to appear. Later the memory fails and there remains little mental activity, the patient lying regardless of his surroundings.

Speech defects

Tremors are amongst the earliest physical signs. They start in the tongue and spread to hands and feet. The speech is jerky and slurring, as also is writing. Mental deficiency further affects spoken and written speech. Pupillary abnormalities are very common, unequal and irregular pupils are the usual phenomenon, complete Argyll Robertson pupil being rather uncommon.

Paresis.

The pyramidal tracts are involved leading to extensor plantar reflex and exaggerated deep reflexes. Attacks of congestive hemiplegia or monoplegia occur with some recovery but ultimately lead to more and more weakness. The control of urinary bladder and defaecation are also lost. Sexual impotence is also present.

Sometimes tabes dorsalis and general paralysis of insane may occur together, and then a mixture of the features of both the disease may be seen. Mental changes are as a rule mild in these cases.

C.S.F. reaction.

The cerebrospinal fluid and the blood give positive Wassermann reaction and Lange's colloidal gold test shows paretic type of curve. Leucocytosis occurs and proteins are increased in the cerebrospinal fluid.

Tabes Dorsalis:—It is the commonest form of nervous-syphilis due to degeneration of posterior columns of the spinal cord. There is a large variety of symptoms, the commonest features are lightning pains.

Sensory disturbances.

Visceral crises.

Objective disturbances of sensation, loss of tendon-reflexes, incoordination, disturbance of pupillary reflexes, in particular the Argyll Robertson pupil and impairment of bladder control are frequent. Less common are visceral 'crises' (acute disturbance of motility of certain viscera), gastric crises and less commonly rectal and vesical, changes in joints (Charcot's disease of joints), perforating ulcers of skin.

Syphilitic progressive muscular atrophy:—The condition closely resembles muscular atrophies of any other origin. The lesion is a degeneration of the anterior horn cells being most pronounced in the cervical region. The atrophy is seen to start in the upper limbs and there may be present some sensory symptoms of tabetic origin. The lower limbs often show spastic paralysis. The condition is steadily progressive.

Acute syphilitic transverse myelitis:—This condition is characterized by dramatic onset of complete paralysis of both the lower limbs, accompanied by paralysis of bladder and rectum, following within six to forty eight hours of onset of pain around the waist. The usual site of the lesion is the eighth spinal segment, where over one or two segments, there is intense infiltration with small round cells.

(iii) Congenital syphilis

Incidence of congenital syphilis.

It is during the first half of pregnancy, that syphilis is usually transferred to the fœtus, and adequate treatment of the mother before this usually prevents foetal infection. The classical sequence in an infected woman is first a series of miscarriages, then still-births at term, then live infants showing signs of the disease at birth, and lastly infants

who appear healthy at birth but who develop signs of syphilis at various ages from one month to many years. Healthy infants may alternate with syphilitic ones. It is the opinion of many eminent pediatricians that congenital syphilis is a comparitively rare disease, inspite of the frequency of the infection among parents.

The great majority of syphilitic infants born alive, show signs of the disease in the first year. The infants blood at birth reflects the condition of the mother's blood and may give positive serum reactions even though the infant be not infected.

Marasmus.

One of the earliest manifestation is wasting or marasmus, and anaemia. Rashes of the skin and mucous membranes are common and appear about one month after birth. The macules are most abundant on the napkin area, around the mouth and nose, on the palms and soles, and at the mucocutaneous margins. Ulcerative fissures may form, leaving radiating scars, which are of great help in diagnosis.

Skin rashes.

The hair of the scalp may be shed extensively, especially over the back and sides, and loss of the eyebrows is highly suggestive of syphilis.

Mucous patches.

Snuffles.

The mouth may show mucous patches, and later in life, perforation of the palate may occur. There may be leucoplakia of the tongue and the fauces may be ulcerated. A chronic nasal catarrh popularly known as 'snuffles' is common and this results in the well known saddle nose caused by the falling in of nasal arch. Involvement of the larynx produces the hoarse cry which together with the snuffles, is characteristic.

Condylomata round about the anal region are not uncommon, and are due to neglect of treatment in long standing cases.

Skull lesions.

The skull frequently shows prominent bossing over the frontal and parietal bones, giving rise to the 'hot-cross bun' appearance of the head. Cranio-tabes may be present: little areas of the skull where the bones are not hard but yielding and parchment-like, especially behind the mastoid. Syphilitic epiphysitis of the long bones is another common manifestation, and occurs early between the sixth week to sixth month. It tends to affect the upper limbs far more than the lower, and is usually symmetrical. The ends of the affected bones are tender and enlarged, and an apparent paralysis is often present. This is due to pain and the child may scream if the limbs are handled. The radiographic appearances are diagnostic.

Lesions of long bones.

Symmetrical painless synovitis may appear in the knee joints at about the age of six years, producing what are termed as 'Cluttons Joints'.

Hutchinsons and Moons teeth.

The permanent teeth may show characteristic changes, the upper and central incisors being most commonly affected. These teeth are spaced out and have wider bases than cutting edges. The cutting edges are rounded off and the centre of the edge is occupied by a crescentic notch forming the Hutchinsonian teeth. Moon's teeth are dome-shaped first molars, the central part having failed to develop and the sides having fallen together. They are diagnostic of congenital syphilis. These changes appear in the permanent teeth only and not in the milk dentition.

Eye lesions.

Eye affections are responsible for a large proportion of blindness in infancy. Choroiditis and iritis often occur in the early months. Interstitial keratitis is common after the first year. The cornea gradually becomes opaque in a patchy manner and after some time, inspite of energetic treatment the other cornea becomes similarly affected. The opacities may become vascularized, and a pink patch, the classical 'salmon patch' is produced. Later the cornea may start bulging or may become ulcerated.

Otitis.

Otitis media may occur in connection with the snuffles. The discharge is very infective and the whole middle ear and labyrinth may be destroyed and the child become a deaf-mute.

Hepatomegaly.

The spleen is frequently enlarged. The liver may be considerably enlarged with a diffuse gummatous hepatitis, and there may be slight jaundice. Paroxysmal hæmoglobinuria is occasionally met with.

A syphilitic orchitis causing a painless enlargement of the testis is fairly commonly met with.

(2) Treatment of Acquired Syphilis

In America the interests of individual who suffers from it are considered less important than the community in which he lives and threatens to infect. Short courses of intensive therapy have been recently devised to get the disease quickly under control but these carry a high mortality rate. On the other hand if treatment lasts a long time, many patients do not complete the treatment and

remain a source of danger to the community. A middle course would appear to be the best. The old conservative methods of treatment and those introduced during recent years have been briefly described in the following pages:—

Importance of early treatment.

The most critical period in syphilis is the first few weeks and adequate treatment begun while the infection is still in the seronegative stage results in complete cure. Delaying the treatment until the patient has developed a positive blood reaction, reduces the patient's chances of being cured by about 20 per cent. From the standpoint of public health protection, early and energetic treatment is essential. Most syphilitic infections are transmitted by persons during the first two years of their infection. The maximum effort in the treatment of syphilis should be in the early stages to control infectiousness and to prevent development of the destructive lesions of late syphilis, and of syphilis in pregnancy to prevent congenital syphilis.

Patients should never be treated merely on the basis of suspicion of syphilis. The diagnosis should be supported by demonstration of the Treponemes, or positive serological reactions supplemented by evaluation of physical signs.

(i) Chances of cure

Cure of syphilis is usually meant to imply that the patient becomes and remains symptomatically well for a lifetime that he is incapable of transmitting the infection to others and that the blood and spinal fluid become and remain serologically negative.

Biological cure.

Biological cure implies complete eradication of treponemes and co-existence of symptomatic and serological cure. It can be most readily attained if treatment is begun during the first six months of infection.

Serological cure.

Serological cure means only that the blood and spinal fluids become and remain negative; it does not necessarily imply biological or symptomatic cure. Symptomatic cure means that the patient becomes and remains well and non-infectious as far as syphilis is concerned, for the rest of his life even though the serological reactions do not remain negative.

Spontaneous cure.

Without treatment, 30 per cent of patients with early syphilis may be expected to develop spontaneous cure; 30 per cent develop latent syphilis, 15 per cent develop benign late syphilis and 25 per cent develop serious late syphilis.

Influence of early treatment on cure rate.

Proper treatment of early syphilis completely alters this picture. In seronegative primary syphilis 100 per cent of biological cures may be attained. In seropositive primary and early secondary syphilis, the cure rate varies from 70 to 95 per cent. In late syphilis, biological cure is unattainable. In benign late syphilis the probability of symptomatic cure ranges from 60 to 95 per cent and of serological cure 30 to 40 per cent. In simple aortitis, symptomatic cure occurs in 70 to 80 per cent, with serological cure in 30 to 40 per cent. When aortic aneurysm is present, arrest of progression of the lesion may be attained.

In syphilis of the central nervous system, biological cure is not to be anticipated. In early involvement of the nervous system, symptomatic cure occurs in 70 to 90 per cent, and serological cure in 85 to 95 per cent. In diffuse, late central nervous system syphilis, symptomatic cure ensues in 60 to 80 per cent and serological cure in 30 to 40 per cent. In tabes dorsalis and general paresis, the best to be hoped for is symptomatic cure.

Early syphilis.

The term 'early syphilis' embraces all forms of the disease developing within the first four years after infection. It includes the primary lesion and secondary lesions, such as rash on the skin and mucous membranes, mucous patches and syphilitic warts. Coincident with these lesions there are lesions in deeper structures including the liver, spleen, heart and brain.

Late syphilis.

Late syphilis includes all types of the disease occuring more than four years after infection. Its manifestations embrace those forms of the disease known as tertiary syphilis. The principal lesions are gummas of the skin, mucous membranes and organs; hepatic syphilis, neurosyphilis, cardiovascular syphilis and syphilis of the bones.

(ii) Treatment of early syphilis

The standard conservative prolonged treatment routines with alternate administration of blocks of arsenicals and bismuth are well adopted to treatment of early syphilis. Stokes et al, commenting on the "official" long term system, conclude that it is effective and will achieve all that the newer shortened systems will, and with a very much greater margin of safety. They further add that the conservative systems are still the back bone of modern practice.

Alternate block method.

The alternating blocking method consists of alternating courses of neoarsphenamine or allied preparations, and courses of bismuth. The first block consists of a suitable arsenical, such as neoarsphenamine, sulpharsphenamine or mapharside, ten to twelve injections in 8 to 10 weeks, and following this with bismuth for six weeks.

Overlap block method.

The overlap block method shown in the table below, is a modification of the alternating block method, is somewhat more intensive, and is preferred by many clinicians. Alternating blocks of mapharside and bismuth are given as in the preceding method; but the arsenical and bismuth are given concurrently for 2 or 3 weeks at the end of each course.

Treatment of early syphilis Standard Long-time Method

Time in Weeks		
0-	*week* 0 *to* 10	
5-	Neoarsphenamine	
10-	or Mapharside	
	10 weekly injections I.V.	
15-		*weeks* 8 *to* 18
		Bismuth
20-		10 weekly injections I.M.
25	*weeks* 16 *to* 26	
30-	Neoarsphenamine	
	or Mapharside	
35-	10 weekly injections I.V.	
		weeks 24 *to* 36
40-		Bismuth
		12 weekly injections I.M.
45-	*weeks* 34 *to* 44	
50-	Neoarsphenamine	
	or Mapharside	
55-	10 weekly injections I.V.	
		weeks 42 *to* 58
60-		Bismuth
		16 weekly injections I.M.
65-	*weeks* 56 *to* 66	
70-	Neoarsphenamine	
	or Mapharside	
75-	10 weekly injections I.V.	
		weeks 64 *to* 80
80-		Bismuth
		16 weekly injections I.M.

Alternating therapy is then continued with alternate overlapping blocks of 10 weekly injections of arsenicals, and 16 weekly injections of bismuth, and is terminated with a course of bismuth.

Potassium Iodide may be given concurrently with each course of bismuth injections, but its use has been abandoned by many syphilologists.,With the conservative routine of syphilo-therapy, the fatality rate is less has 1 in 3000.

The dosage of neoarsphenamine recomomended for use in the above plan are 0.6 to 0.75 gm. weekly for men, and 0.4 to 0.6 gm weekly for women. Mapharside is recommended by the manufacturers (Parke Davis and Co.) to be used in doses 40 to 80 mgm. weekly, depending on the weight of the patient.

Bismuth injections in the above course are given in doses of 0.2 gm of bismuth metal weekly.

The superiority of the continuous treatment plan, over the older schemes of interrupted therapy, particularly in early syphilis, is no longer in doubt. It is now generally admitted that the continuous treatment of early syphilis is so far superior to any intermittent or irregular system, as to leave no room for comparison. In early active syphilis, all conservative routines call for at least 30 injections of arsenicals and from 30 to 36 injections of bismuth.

With any long-term plan for treatment of early syphilis, the average patient should be seronegative by the 16th week of therapy. If the blood still remains positive at the 24th week of treatment, the spinal fluid serology should be examined. If after the 26th week of treatment, weak positive serological reactions appear among negative blood tests, infectious relapse or neurosyphilis should be suspected.

(iii) Modern intensive therapy

These methods are coming into increasing use, and are most suitable for patients with early syphilis, who have received little or no previous anti-syphilitic treatment. Of these methods the two most suitable for general clinical and hospital use are:—(1) The U.S. Army plan which is outlined by Turner and Sternberg (1944), and (2) The Eagle twelve week schedule (Eagle and Hogan 1943). It must be cautioned here, that until further experience has accumulated proving their efficiency and safety, the Eagle Plan, and the U.S. Army Plan for intensified ambulatory treatment of early syphilis are not recommended for general use, except where physicians feel that patients will otherwise fail to complete the standard fiftytwo to eighty weeks course of arsenicals and bismuth now in general use.

The U.S. Army plan.

The U.S. Army Plan course extends over a period of 26 weeks. Neoarsphenamine or mapharside is given in 20 injections twice weekly during the first ten weeks, omitted during the next six weeks, and another twenty twice weekly injections are given during the last 10 weeks. Weekly injections of bismuth are given during weeks 0 to 5, omitted during weeks 5 to 10, given again during weeks 10 to 16, omitted during weeks 16 to 21 and finally given again during weeks 21 to 26.

This plan calls for forty injections of neoarsphenamine in doses of 0.6 gm or of mapharside in doses of 60 mgm; and sixteen injections of bismuth during the 26 weeks. Results are probably as good as with standard routines, and the risk to the patient is only slightly greater. Eagle estimates that mortality with this method of treatment ranges from 1 in 1,000 to 1 in 3,000, the margin of safety being 8 as compared with a margin of 10 for standard therapy.

The eagle plan.

The twelve week Eagle Plan extends over twelve weeks during which period a total of 36 injections of neoarsphenamine or mapharside are required, 3 injections being given each week. In addition to arsenicals, weekly injections of bismuth salicylate (0.2 gm equivalent to 0.13 gm of bismuth metal) are given. This undoubtedly helps in producing a cure. Three weekly injections of mapharside alone produced fewer cures but combined with bismuth more than 80 per cent of patients were cured in 9 to 12 weeks. Encephalopathy, dermatitis, jaundice and fall in polymorphonuclear leucocytes rarely occur with intensive therapy. Results are said to be good in seronegative and seropositive primary syphilis and in secondary syphilis. However as the curative dose of the arsenical is given in a much shorter time than by other methods of ambulant therapy, the risk of serious ill effects is increased. The estimated mortality with the Eagle Plan is 1 in 400 to 1 in 1000. Thus, this plan should only be used only with full recognition of its potential danger to the patient.

Massive dose arsenotherapy.

Massive Dose Arsenotherapy. Intensive therapy of early syphilis with heroic doses of arsenicals is best exemplified by the five day drip method introduced by the Mt. Sinai Hospital (New York) group (Hyman, Chagrin, Rice and Leifer 1939). It consists of giving 200 to 240 mgm of mapharside in 2,000 to 2,400 ccm of 5 per cent glucose solution, by slow intravenous drip (3 cc per minute) over an eight to twelve hour period daily, for five days. A total dose of 1,000 to 1,200 mgm, or the equivalent of twenty standard doses of mapharside, is thus given over a period of five days.

Modifications of intensive drip therapy of early syphilis, using multiple injections by the syringe, have been tried. They call for a total dosage of 1,200 mgm of mapharside over a period of six to twenty days, the average period being ten days. One method consists essentially of giving an injection of 60 to 100 mgm of mapharside once or twice daily and repeating this every day until a total of 1,200 mgm has been given. The U.S. Army adapted a 20-day treatment in which 1.0 mgm of mapharside was given per kilo body weight daily together with 8 doses of bismuth salicylate. In some patients treatment had to be discontinued because reactions occured commonest being fever from 6th to 15th day of treatment; jaundice and encephalopathy rarely occured.

Data at present available on the risk to patients receiving intensive chemotherapy within a one to twelve day period reveal that the mortality is in the neighbourhood of 1 in every 200 patients treated. This death rate, even under expert management, emphasizes the hazard of ultra-intensive arsenotherapy in general practice.

Combination with artificial fever therapy. That heat is inimical for treponema has been shown by the fact that in rabbits scrotal syphilis is cured by repeated heating to a body temperature of 43° C. The heat may be applied, (1) by inducing paroxysms of malaria, (2) by injectiong TAB vaccine intravenously and (3) by raising temperature of the body by application of heat. This form of therapy is useful in cases of progressive paralysis especially of the severe type.

Artificial fever therapy has been considered better than malaria and vaccine therapy, but it requires an expensive apparatus. The advantage is that the temperature can be regulated, its duration of application can be varied as desired. The author, has however, used malaria therapy successfully in India in a number of cases of neurosyphilis without any untoward effects

Artificial fever therapy is not without risk to the patient and his condition has to be carefully watched during its application. Treatment should be stopped if the systolic pressure falls below 100 or pulse rate goes over 150 and respiration rate over 50 per minute, or there is extreme restlessness, vomiting or coma.

Fever therapy has been combined with injections of arsenic and bismuth but has not been found satisfactory in the treatment of early syphilis. Before each application the patient is given an intramuscular injections of bismuth salicylate (4 grains); mapharside in doses of 60 to 100 mgm is given an hour and a half after the fever (105 to 106°F) has been produced, which should be continued for ten hours.

(iv) Penicillin in the treatment of syphilis

Four different types of penicillins have been isolated, F,G,X & K. Of these the most active against syphilis is G; K is least efficacious because it is rapidly excreted. Penicillin is undoubtedly effective against primary and secondary syphilis, but its exact status cannot at present be stated especially in the treatment of chronic acquired and congenital syphilis. It can, however, be said that in the treatment of early acquired syphilis it compares favourably with arsenicals and bismuth compounds. Adequate treatment with this drug may reduce cardiovascular syphilis, neuro-syphilis and other chronic types. On account of the low toxicity of penicillin, an intensive and massive treatment can be given without harm which is not possible with arsenicals. Penicillin has also been shown to be just as effective as arsenicals and bismuth compounds in the prevention of congenital syphilis when given to pregnant women. It is also effective in healing the gummatous lesions of the skin and mucous membrane. It cures cases of early syphilis resistant to arsenicals and bismuth compounds, but is ineffective against Wassermann-fast syphilis. It is particularly useful in cases showing sensitiveness to organic arsenicals. Herxheimer's reactions undoubtedly occur with penicillin, but these can be prevented by regulating the dose in the early stages of the treatment, and often are not serious.

Penicillin G in Syphilis.

The disadvantages of penicillin are the need of frequent intramuscular injections (every 3 to 4 hours) but this may be overcome by giving 3 to 6 hundred thousand units of the drug daily in peanut oil and bees wax or aqueous solutions mixed with vegetable oils, but weekly injections like those of arsenicals and bismuth are not yet possible. Perhaps suitable compounds for this and for oral administration will be discovered in the near future which will make treatment very convenient.

Penicillin in early syphilis (Primary & Secondary). The quick control of infectiousness is a very important consideration. Treatment with arsenicals and bismuth compounds for 1 to 1½ years gives 90 to 95 per cent chance of a biological cure and the results with penicillin therapy should at least be comparable. Different forms of treatment with varying doses and duration have been tried but the optimum dosage has not been definitely determined. As mere healing of the lesions is no criterion, on the basis of serological and clinical relapses, a total dosage of a minimum of 2,400,000 units by intermittent intramuscular injections is considered effective (300,000 units daily for 8 days). This should be combined with daily intravenous injections of 40 to 60 mgm of mapharside. The two drugs undoubtedly have a synergistic effect.

Methods adopted for penicillin treatment are:—

(1) 60 injections of 40,000 units every four hours in ten days., (2) 300,000 units in peanut oil or bees wax daily for 8 days. 40 mgm of mapharside are also given in both (1 & 2) intravenously: (3) 600,000 units in peanut oil and bees wax once a day for 8 days.

Methods.

The patient is carefully observed for 2 to 3 months, weekly clinical examinations being made; serum and cerebro-spinal fluid are examined at the end of this period. In sero-negative primary cases one course is sufficient but in seropositive cases one or more courses with or without mapharside are recommended. After this serologic and clinical examinations should be made twice a year for five years. If after 2 to 4 months of treatment there is clinical relapse or serum is positive, it is no use giving penicillin treatment but treatment with arsenicals and bismuth should be given. If coincident gonorrhœa is present penicillin may mask or delay development of chancre. In such cases the patient should be kept under prolonged observation (Kolmer, 1947).

Ambulatory treatment. The use of penicillin in the treatment of syphilis required hospitalisation of the patient for 8 days. As this is often inconvenient an ambulatory method was devised by Lloyd Jones and Maitland (1945). They give single daily intravenous injections of penicillin for 8 days, the total dose given being 2.4 to 5.0 million units. The relapse rate with this treatment was 4.0 per cent.

The U.S. Public Health service have extensively used penicillin for the treatment of early syphilis and more than 150,000 patients have been treated with various systems. The results suggest that best method so far employed is, the combination of 5 injections of oxyphenarsine hydrochloride (mapharside), 1,200,000 units of penicillin, and 3 injections of a bismuth preparation, given over a period of 10 days.

Mapharside and penicillin.

Another system that is at present extensively employed, is to give, as soon as the diagnosis of syphilis is made, four injections of 0.04 gm of mapharside on 4 *consequitive* days. On the 5th day penicillin treatment is started and 40,000 units are given every 3 hours for 8 days (total 2,400,000 units). On the last day of the penicillin course, treatment with bismuth salicylate in doses of 0.1 gm is started, and ten injections are given at the rate of one every fifth day.

Serological response.

The blood serology of patients who progress satisfactorily, becomes negative on the average from sixty to one hundred and twenty days after the commencement of treatment. Patients should be examined at monthly intervals for the first year, with clinical inspections and with quantitatively titred blood serological tests. In the absence of clinical manifestation and with a blood serological titre falling to or towards seronegativity, the patient is progressing satisfactorily. On the other hand, the absence of quantitative serological response, the presence of serological relapse as indicated by a rising titre, or the appearance of new clinical manifestations, is an indication for retreatment. The patients' spinal fluid should be tested six months after the completion of penicillin therapy. If progress during the first year after treatment has been uneventful and seronegativity achieved, the patient may be checked during the second year every three months. Continued and prolonged observation for a minimum of five years is desirable. If relapse occurs, one may treat the patient again with penicillin utilizing for the second course a total dose at least twice that originally administered and combining with the second course a minimum of six injections of mapharside.

On comparison of the results of various rapid systems of treatment, the results favour penicillin only in the fewer number of reactions it produces. The intensive measures, such as the 5 days drip method or multiple injection technique with syringe, using oxyphenarsine hydrochloride, have a lower rate of failures but the incidence of mild and serious reactions is considerably higher. This seriously minimizes the practical value of the methods.

The value of penicillin by the oral route, and the daily injections of 30,000 units of penicillin, in beeswax and peanut oil are still being investigated. Present experience with oily and beeswax preparations suggest that these methods may be of considerable value in simplifying penicillin therapy by making only one injection in a day necessary, for several weeks, instead of one injection every 3 hours for 8 or 10 days.

Penicillin in the prevention of congenital syphilis.

Results hitherto obtained from the use of penicillin in preventing congenital syphilis, by far excel those with any previously used scheme, and in a series of cases it was reported that only 1 out of a 100 pregnant women so treated, gave birth to a syphilitic child. The most efficient method appears to be the administration of 2,400,000 units in 8 days in the third month and again in the seventh month of pregnancy. Complications are usually not encountered. In the treatment of congenital syphilitic children however, penicillin has not so far proved so highly, efficacious. The benign manifestations such as periostitis or cutaneous gummas respond rapidly, but interstitial keratitis had proved resistant to penicillin therapy.

(v) Therapy of late syphilis

Under the term late syphilis are included latent syphilis, visceral syphilis, neurosyphilis (including tabes dorsalis & paresis) and also cases of syphilis resistant to treatment.

In late syphilis, continuous treatment is desirable but not essential. Heavy metals are given over a long period of time. Bismuth and iodides should precede arsenicals for four to ten weeks. A suggested plan for the treatment of late syphilis by the conservative long-term method is given below:—

Week	*Arsenical*	*Week*	*Bismuth*	*Iodide*
0-4	—	0-6	6 weekly injections I.M.	Daily by mouth
4-12	6 weekly injections I.V.	6-10	——	——
12-16	—	10-18	8 weekly injections I.M.	Daily by mouth
16-26	10 weekly injections I.V.	18-24	——	——
26-34	—	24-36	12 weekly injections I.M.	Daily by mouth
34-44	10 weekly injections I.V.	36-42	——	——
44-72	—	42-54	12 weekly injections I.M.	Daily by mouth
72-82	10 weekly injections I.V.	54-62	——	——
		62-74	12 weekly injections I.M.	Daily by mouth
		74-80	——	——
		80-96	16 weekly injections I.M.	Daily by mouth

Due to the probability of cardiovascular and visceral involvement in late syphilis, reactions must be avoided and cautious use of arsenicals is essential. To avoid therapeutic shock and therapeutic paradox, therapy in late syphilis should always begin with bismuth and iodides. A rest period of eight to ten weeks after one year of continuous treatment probably does no harm in late syphilis, but many syphilologists prefer continuous therapy.

The Co-operative clinical group of U. S. A.'s (Stokes et al 1943) experience indicates that three courses of eight injections each of an effective arsenical such as neoarsphenamine or mapharside, give in alternation with 10 injections of bismuth, without overlap and with continuity extending only through the arsenical phase, constitute an adequate beginning. Continuous treatment with arsenicals and bismuth is then followed by intermittent and prolonged treatment with bismuth alone, which should total including the three ten-injection bismuth courses given with the arsenical phase, not less than sixty weeks of bismuth therapy .

Semi-intensive therapy is suitable for treatment of many cases of late syphilis. The 26 week system used by the U. S. Army for early syphilis is also recommended by them for treatment of latent syphilis, and on theoretical grounds there appears to be no reason why it should not prove satisfactory. Long-term studies of this phase of arseno-therapy have been under way for some time but it is too early for any conclusions.

Penicillin in late syphilis.—The response of the late cutaneous manifestations of syphilis to penicillin therapy has been so rapid and remarkable that, although a considerable period must elapse before its ultimate effect on tertiary syphilis can be evaluated with accuracy, it has been recommended by eminent venereologists that with the exception of gross cardio-vascular disease, all late cases should be given the benefit of penicillin therapy, in addition to whatever other treatment is necessary. The risk of a Herxheimer reaction in late syphilis, however makes it necessary that therapy should in all cases commence very cautiously, and 6 to 10 injections of bismuth with concurrent iodides by mouth every 4 or 5 days should always precede penicillin or even arsenicals. The dosage of penicillin employed should total from two to four million units given 3 hourly over a period of 8 days.

It is not known whether penicillin penetrates into the syphilitic lesions as well or better than arsenicals and bismuth compounds or whether clinical and serologic improvement is more or less readily produced or whether a combination of the two is more effective than penicillin alone. Kolmer (1947) from his vast experience has suggested the following plans of treatment:—

(1) 180 injections of penicillin in 30 days, 10,000 units being given in aqueous solution every four hours totalling 1,800,000 units. (2) Or penicillin as above but in combination with five doses of 40 mgm of mapharside intravenously alternating every three days and five doses of bismuth over a period of 30 days. (3) Or intramuscular injections of 300,000 units of penicillin in peanut oil or bees wax or water-oil emulsion once a day for 30 days combined with mapharside 40 mgm every 5 days (total 6 doses). (4) A rest period of two months and then the whole course is repeated. This is carried on for a year or longer so that at least four courses are given in chronic latent syphilis and 4 to 6 courses in chronic symptomatic syphilis unless otherwise indicated by serologic and spinal fluid examinations.

Cases resistant to arsenicals and bismuth generally respond to 60 doses of 40,000 units every 3 or 4 hours. In sero-resistant cases there is probably a persistent treatment-resistant focus (Kolmer) in the body and penicillin has not proved effective in these cases. Optic neuritis has improved with doses of 2 to 4 million units and progress of primary optic atrophy has been checked. In latent syphilis penicillin is an excellent substitute for arsenical and bismuth compounds. As many as a hundred injections of 40,000 units have been given with improvement in serologic reactions.

Penicillin is well borne in pregnancy when administered to infected pregnant women in adequate doses (1,200,000 to 2,400,000 units intramuscularly); normal infants are born in more than 90 per cent after this treatment (arsenicals also do this). The drug is therefore transmitted to foetus through the placenta. Cases of early congenital syphilis also improve under penicillin treatment and about 50 per cent become sero-negative.

Prophylaxis of syphilis. Prophylaxis in man with a single dose of penicillin,however large, should never be tried as it is likely to suppress and not eradicate the disease. The patient is thus placed in a very unfortunate position and uncertainty may persist for years. If prophylaxis is to be given at least 20 intramuscular injections of 40,000 units in peanut oil and wax daily for 3 or 4 days in succession should be given.

Penicillin in Neurosyphilis.—Dramatic improvement has been reported in both the clinical condition and the cerebrospinal fluid in acute meningo-vascular syphilis, though there is a risk of severe Herxheimer reactions such as acute transverse myelitis if preparatory preparation with bismuth injections is not done. In tabes dorsalis the progress of primary optic atrophy is arrested and the cerebrospinal fluid improves. There is also some improvement in other symptoms such as lightening pains.

In dementia paralytica the drug should be used in combination with arsenicals, bismuth or fever therapy. Reports of results are conflicting, but it is generally agreed that some improvement occurs in the spinal fluid of more than 50 per cent of the cases treated.

In asymptomatic neurosyphilis the results are excellent, the spinal fluids becoming normal in the majority of cases treated with penicillin alone. Preparatory bismuth treatment should however always be given, and it is advisable to supplement the treatment with arsenicals.

2. Yaws

Yaws or framboesia is an infectious, contagious tropical disease of non-venereal origin; it is characterised by an initial cutaneous papillary lesion the "mother yaw" and is followed by a multiple, papillomatous, granulomatous eruption of the skin; sometime late destructive lesions, especially of the skin and bones occur. It is caused by the *Treponema pertenue,* which is morphologically indistinguishable from *T. pallidum* of *syphilis.* The organism is present in large numbers in discharges from lesions.

Yaws essentially occurs in the tropics and it does not spread when introduced to temperate zones. In cold climate in the tropics it occurs in a modified form. The infection is aquired innocently in childhood through an abrasion in the skin. It is common in the West Indies, tropical America and throughout equatorial Africa. It is prevalent in Indonesia, Ceylon, Malay States and Burma; it is comparatively rare in India, being occasionally seen in Assam and parts of Bihar.

Various views are held on the relationship of yaws to syphilis but perhaps most conservative view in this connection is that the virus of yaws is a modified virus of syphilis; a less virulent one, producing a disease which has been modified through the epidermis in tropical peoples, and by their habits and the climate and conditions under which they live. Syphilitic lesions occur in the skin, bones, muscles, the nervous system and the cardiovascular system. The lesions of yaws are confined to the skin and later to the bones and the disease is not congenital. There is much convincing evidence that an individual who has acquired immunity to yaws, is also immune to syphilis, and vice versa.

Yaws is readily communicable, and man and monkeys may be successfully infected with material from one of the lesions of the skin. Auto-inoculation may occur early in disease and cause spread of lesions. There is great thickening of epidermis with degeneration of cells; there is exudation of serous fluid and leucocytic infiltration in circular masses as in miliary abscess. A common late lesion is gummatous ulceration of the skin. Nasal, buccal and conjunctival mucous membranes may become affected. The disease is not

venereal and natural infection in man occurs most commonly through abrasions in the skin by direct contact with an infected case. Flies especially *Hippealates* are generally recognized as potent factors in spread of the disease.

Clinical aspects.

Symptoms in Initial stage.—Poor native children, without clothing and with frequent skin wounds, are the individuals most commonly affected. During the incubation period which averages 3 to 4 weeks, digestive troubles, nocturnal headache, joint pains and an irregular fever may be noted. These usually abate with the appearance of the initial papule (mother yaws) at the site of inoculation. This lesion which is almost always extra-genital may be single or several papules may be grouped together, and the regional lymphatic glands are enlarged and tender. It is not painful but bleeds easily.

The Secondary or generalised stage.—From six weeks to 3 months after appearance of the initial lesion, which may have dried up or which may still persist, there occur again, headache, joint pains and fever, and the secondary generalized eruption appears. This eruption is made up of papules similar to the primary papules, and the distribution is most frequent on the muco-cutaneous junctions, the face, neck, arms, legs, buttocks, palm of hands or soles of foot. The generalized stage lasts from 3 to 4 months to 2 or 3 years. The yaws tubercles come out in successive crops. They are not painful but in some cases itching is troublesome.

Four groups of secondary skin lesions have been described:—(*i*) Roseola, which are fleeting and often go unnoticed. They appear about twenty days after the primary sore. (*ii*) Pianides, appearing in interval between roseola and generalised eruptions. (*iii*) Mucosal lesions on the lips and the tongue in plaques resembling those of syphilitic leucoplakia. (*iv*) Piamomas, are vegetating papillomatous lesions oozing but not desquamate.

Tertiary lesions of yaws are gummatous nodules and deep ulceration. They may appear as destructive lesions of the naso-pharyngeal region, (tibia) dactylitis, osteitis, periosteal nodes, juxta-articular nodes and gangosa (Rhinopharingitis mutilans). These may set in years after an attack of yaws.

Differentiation Between Yaws and Syphilis

Das Gupta has summarised the following main points in which yaws differs from syphilis: (*i*) Primary sores are practically always extragenital in position; (*ii*) secondary lesions never or only very rarely affect mucous membranes; (*iii*) alopecia and eye symptoms are absent; (*iv*) in the tertiary stage, vessels, organs, and nervous system are not affected; (*v*) the disease is not transmitted congenitally, and *transmission* is affected by direct contact; (*vi*) infection with syphilis does not protect against infection with yaws; (*vii*) yaws is more easily transmissible to experimental animals than syphilis; and (*viii*) mercury does not appear to have any effect on yaws.

Gangosa.—The mucous membrane becomes honeycombed with deep ulcers underneath. The ulcerating processes progress rapidly, becomes secondarily infected and destroy bones and soft parts; eventually the whole of the nose and septum disappear. The larynx is not affected. Gangosa occurs in Fiji, West Africa, and Congo in places where treatment is not available.

Goundou occurs where yaws is common and follows closely the secondary stage. There is persistent headache and thin nasal discharge accompanied by enlargement of the nasal processes of upper maxilla downwards and outwards till a projecting tumour as large as a fist forms. As the exostoses grow, the nasal passages are obstructed and vision is interfered with. Goundou is usually bilateral. Treatment consists in surgical removal.

Juxta-articular nodes.—The nodes are composed of irregularly distributed bands of connective tissues without elastic fibres and with poor blood supply. Some observers do not connect it with yaws nor syphilis. The tumours vary in size up to a golf ball and are very hard; the skin over them is moveable at first, but later becomes fixed; they are not painful and never suppurate. They are located in the external surfaces of extremities and near the joints. They may become irritated and behave as neoplasms. They may be mistaken for tumours produced by *Onchocerca volvulus.*

Treatment consists of excision of the growths if they are troublesome.

Diagnosis.—Staining of the juice expressed from yaws tubercles by the Indian ink method, with Giemsa stain, or under dark-ground illumination is the best diagnostic procedure. The Kahn and Wassermann reactions are positive.

Prophylaxis and Treatment.—prophylaxis measures depend on the adoption of measures to prevent infection by direct contact with yaws cases, which should be isolated and given prompt treatment with arsphenamine.

In endemic areas, fly protection measures should be generally adopted, and abrasions, should be protected by suitable dressing. Clothing contaminated with the discharges of yaws is well known as a disseminating factor, and should be boiled or disinfected.

Arsenicals.

The effect of arsphenamine in the treatment of yaws, provides a most remarkable example of the specific action of a drug in therapy, and in early cases one single dose frequently effects a complete cure, thus almost approaching Ehrlich's dream of a 'therapia magna sterilans'. In the treatment of early yaws, although as stated above, one single injection often effects a cure, two or three doses are advisable in order to prevent relapses. Neo-arsphenamine is given in doses of 0.6 gm to 0.9 gm intravenously, or Mapharside may be used in doses of 0.06 gm for adult males, and 0.04 gm for adult females. For children between the ages of 2 to 10 years, doses of 0.3 gm of neo-arsphenamine or 0.03 gm of mapharside, are suitable. For infants under 2 years of age the corresponding doses should be 0.1 gm and 0.01 gm respectively. Intramuscular injections are to be prefered in very young children. Usually within ten days granulomata are absorbed.

In the treatment of tertiary yaws, more prolonged treatment is necessary, and the following course is generally recommended. During the first four weeks, four weekly injections of neo-arphenamine or mapharside in the above mentioned dosage, are given and bismuth subsalicylate injections 0.2 gm are given on the same days. This is followed by another four weekly injections of arsenical alone, and the course is terminated with another 8 weekly injections of bismuth subsalicylate.

In the writers experience, yaws occuring in India is a comparatively mild disease, and most cases respond readily to one to three injections of a suitable arsenical or bismuth preparation. Doses of 0.19 gm of neosalvarsan per kilo produced clinical cure in two injections in 93.3 per cent of cases in Fiji. In other places more injections were required.

Many bismuth preparations are equal in efficiency to neo-arsphenamine, according to most authorities. Sodium potassium bismuth tartrate, bistoval, bismuth arsenicals, bismuth salicylate and metallic bismuth in oil are used. Other drugs used are stovarsol, carbarsone and halarsol (oxyameno-phenyl dichlorarsine).

The use of 100,000 units of penicillin in 4 doses in nine to twelve hours is as effective as many incomplete courses of arsenicals given over a longer period. It will cause the healing of secondary lesions, and thus render the patient non-infectious, at least for the time being. The skin and bone lesions respond more slowly, but these cases had not healed previously despite prolonged and repeated arsenical and bismuth treatment. Treatment with 15,000 units intramuscularly every four hours for seven days are stated to have produced a cure, the lesions clearing up by the end of a week, clinical cure being complete. Serological reactions however remained unchanged. Penicillin is likely to be effective in the treatment of pinta which is now known to be due to *T. carateum or T. herrijoni* (closely related to *T. pallidum*).

3. The Relapsing Fevers

Two different types of Relapsing fever are recognised, and though caused by closely related spirochaetes, they differ from each other in many important aspects such as epidemiology, mode of transmission, severity of clinical manifestations, and mortality rates.

(1) Louse Relapsing Fever

The louse-borne or Epidemic Relapsing fever, is caused by *the Borralia recurrentis* and allied varieties (*B. carteri* of Indian relapsing fever, and *B. berberi* of North Africa).

The disease is widespread throughout the world and particularly in India, where the disease occurs in epidemic form throughout the country except in Bengal, Assam, and Orissa. The infection is conveyed directly from man to man by the human louse *Pediculus humanus,* either through a bite wound, or a scratch wound being contaminated by infective material from a crushed louse.

B. recurrentis is a spiral organism varying from 9 to 10 μ in length and 0.3 to 0.4 μ in breadth; and is found in the blood during the paroxysms of fever. Sometimes two or more spirochaetes are found attached end to end. The organisms are most plentiful after the first 2 days, and may be easily stained by any of the modifications of the Romanowsky stain, such as Giemsas or Lieshmans.

One attack confers immunity for a period of one to two years.

Pathology.

The skin may show jaundice and peticheal hæmorrhages. The internal organs are congested and hæmorrhagic. The liver and spleen are enlarged and may show areas of necrosis and infarcts. Fatty degeneration of the liver is often a prominent feature. The brain is congested and hæmorrhagic; the heart may show cloudy swelling, and the bone marrow is hyperæmic. Spirochaetes are plentiful in all the organs, especially in the reticulo-endothelial cells.

Symptomatology.

In Northern India, the incidence rises in autumn, reaching its maximum in March or April, after which there is a rapid decline.

The disease generally comes on very suddenly with chill, severe headache, dizziness, and great prostration. Severe pains in the back, arms and legs, are prominent features. The temperature rapidly rises to 104 or 105°F or higher; the pulse is rapid and the patient may be delerious. Jaundice may appear if the fever has lasted long, and an erythematous eruption is sometimes seen over the trunk and limbs.

The fever generally lasts from three to six days, after which it falls by crisis, and is accompanied by profuse sweating. This is followed by an afebrile period lasting from 3 to 10 days, and then there is another attack of fever similar to the initial attack, which again terminates by crisis. There are usually four or five such relapses, the febrile periods tending to become shorter, and the remissions longer. Pneumonia, diarrhoea, dysentry, transient paralyses or heart failure may occur as serious complications.

Meningitic forms of louse-borne relapsing fever have been described in North Africa which respond easily to treatment with arsenicals.

Prognosis.

According to Rogers and Megaw, the mortality in India varies from 1 to 50 per cent in different epidemics, the average being about 10 to 15 per cent. If proper treatment with one of the arsphenamines is given promptly, the outlook is very good and the mortality is practically nil.

The patient should be kept strictly in bed throughout the disease, and for many days after. The bowels are kept open, plenty of fluids are advised, and a liquid but nutritious diet prescribed. *Treatment.*

One injection of neoarsphenamine, mapharside or one of allied compounds, will terminate most attacks, but relapses are common unless 2 to 4 injections are given. The drug should never be given just before the crisis is expected, as there is a very real danger of fatal collapse, due presumably to liberation of large amount of toxic material from the large number of spirochaetes destroyed. Nor should arsenical drugs be given if cardiac, renal or hepatic disease is present. The best time to administer the drug is soon after the beginning of a spell of fever, or at the height of a paroxysm. The dosage generally employed is, neoarsphenamine, 0.3 to 0.9 gm for adults, and mapharside, 0.04 to 0.06 gm. Stovarsol by mouth has also been reported to be very effective, in doses of 4 grs (0.25 gm) 5 or 6 times a day.

Bismuth compounds have been used in resistant cases with good results.

(2) Tick Relapsing Fever

Tick-borne relapsing fever, is a non-epidemic disease, which is epizootic in certain rodents such as rats, mice, etc., and is only incidentally transmitted to man by the bites of certain ticks. The infecting organism is *B. duttoni* which is morphologically identical with *B. recurrentis.* The ticks involved in the transmisstion of the disease belong to the genus *Ornithodorus,* and the tick responsible for the transmisstion in India and Africa is *O. moubata,* though other varieties are incriminated in other countries, *e.g., O. papillines O. tholozane* and O. *lahorensis* in Iran and Northwest Asia; *O. taloje* in South and Central America.

The disease is predominantly a "locality" disease, and the infection persists in the ticks of certain houses and camping places. The progeny of infected ticks are also infective. Ticks are very difficult to destroy, as they usually live in cracks of walls, ceilings and floor.

Tick-brone relapsing fever is generally a less severe disease than its louse-borne prototype, and the mortality is usually below 5 per cent. The spells of fever are shorter in duration, but more intense and usually last 2 to 4 days. There are usually more relapses, commonly 5 to 6, but the whole disease runs a shorter course. Spirochaetes are not so numerous in the blood, and certain complications such as diarrhoea and dysentery, paralyses and iritis, are more commonly encountered. *Symptomatology.*

Treatment is on the same lines as for louseborne relapsing fever, but generally speaking, larger doses of arsenicals are required to overcome the infection. *Treatment.*

Penicillin has been tried in the treatment of relapsing fever in doses ranging from 100,000 units in 24 hours, to 900,000 units in 72 hours, in a series of 11 patients, 5 relapsed; in another series of 18 patients in which the treatment was combined with a single maximum dose of NAB, there were 7 relapses. The results indicate that neither penicillin nor NAB, either alone or in combination, is effective in preventing relapses of African tick fever.

In cases of louse-borne relapsing fever, however, in a series of 27 patients treated with total dose of 480,000 units for adults and 360,000 units for children, the spirochætes disappeared within 24 hours and none were ever found again after 48 hours. All patients showed a rapid clinical recovery and there were no relapses. The results with neoarsphenamine were also equally satisfactory.

Tick-borne relapsing fever has been reported in India but its being tick-borne has only been a surmise, except in Kashmir where *O. crossi* has been shown to be the carrier of infection. *O. moubata*—so far as is known has not been proved of relapsing fever in India.

In Kashmir the disease is mild, the mortality is nil, complications such as diarrhœa, dysentery, paralysis and iritis are unknown. In untreated cases, there are generally 5 to 8 relapses. Pencillin has no effect in any dosage. Ninety per cent of cases are cured by 1.0 gm of NAB, the rest require a third injection of 0.6 gm.

In Kashmir the vector and the disease are universally spread and not delimited to any particular endemic focus. The incidence is high in summer (because the ticks are more active) but cases occur throughout the year.

DDT and gammexane as dust on the floor and oily solution in the case of DDT and watery suspension in case of gammexane, have proved effective in the control of these ticks.

4. Rat-bite Fever

Rat-bite fever is a relapsing fever occurring in people bitten by rats infected with *Spirillum minus* (*Spirillum morsus-muris*). The disease is as widespread as the rodent host in its distribution and cases have been reported from practically all parts of the world. It is, however, much more prevalent in Japan than in other countries. The organism is variable in size, the usual being 2 to 6 μ long with a delicate flagellum with two to six curves, but much longer forms up to 20 μ are occasionally seen, especially in cultures. They are found at first in the blood of infected mice, rats or guinea pigs and then become disseminated in the tissues, particularly around the lips, tongue and nose. They have not been found in the saliva and transfer of infection to man would appear to be through some break in the mucous membrane around the mouth.

Another form of rat-bite fever, which is quite common is that caused by *Streptobacillus moniliformis,* and is transmitted to man not only by the bites of rat, but also by cat-bites.

Symptomatology.

Four clinical types have been differentiated:—(1) With general symptoms predominating. (2) With local symptoms predominating. (3) A type with severe pains. (4) A type with neurological manifestations, even paralysis.

The incubation period varies from 5 to 40 days, the average being less than 10 days. Following this period during which time the wound of the rat-bite heals, there is usually a sudden onset with headache, nausea and marked weakness; the pulse is rapid and weak. The scar of the wound becomes inflamed and even necrosis may occur. The regional lymphatic glands are swollen and tender and there may be œdema of the hands and legs. Fever rises rapidly to 101 to 102°F and within 2 or 3 days may reach 104°F or more, and then remains high for another 2 to 3 days. After this it falls rapidly to normal, accompanied by profuse sweating. The temperature remains normal for a few days, during which time the local swelling and inflammation subside. Joint pains, sensory disturbances and symptoms of nephritis may be noted. After a few days of normal temperature the fever again comes on, later to disappear and re-appear. The successive paroxysms are usually of less severity and separated by increasing intervals. A syphilis-like eruption may occur but Wasserman reaction is usually negative.

The primary wound may become infected with cocci, bacilli and streptothrix and give rise to fatal septicaemia. There may be as many as 12 of these febrile waves and the course of the disease may extend over several months. There is an eosinophilia and during the febrile phases a leucocytosis of 12 to 15,000 is present.

In infections caused by the *Streptobacillus moniliformis,* the initial rat-bite wound heals rapidly, but marked constitutional symptoms appear 2 or 3 days after the bite. Special features are severe arthritis and painful nodules in the muscles.

Diagnosis.

Diagnosis.—From history of rat-bite. The blood should be examined for spirilla by dark ground illumination during the early febrile periods. Should this prove negative, guinea pigs, white mice or white rats may be inoculated with blood or gland juice, care being taken that the animal is not already infected. Sprillum infection is common among guinea pigs in India. The Wassermann reaction is usually negative though in a few cases positive reactions may be met with.

Prognosis.

One attack generally confers a lasting immunity in both man and animals. Bacteriolytic antibodies have been demonstrated. In Japan there was mortality rate of 10 per cent but since the advent of arsenical therapy deaths are rare. Rat-bite fever has been used in the treatment of general paralysis.

Prophylaxis.

Effective prophylaxis depends on rat destruction and prevention of contact with rats, especially in darkened areas where bites are more likely to occur.

Treatment.

Treatment.—One or two injections of neoarsphenamine or some of its derivatives will usually cure the disease and the ordinary doses of 0.3 to 0.6 gm are usually sufficient. The injections should be given early in each spell of fever and not at the end of the paroxysm, in the same way as in relapsing fevers. Since recurrences are common (especially in Japan) when less than 3 injections are given, a course of 3 to 6 injections is advisable. Otherwise treatment is symptomatic. Aspirin relieves severe joint pains. Strychnine may be given for weak heart. Local treatment of the wound or even excision are of little value though it is advisable to cauterize the wound with phenol or tincture iodine.

Antimony preparations, such as stibosan have been successfully used by some workers.

While infections with *S. minus* respond well to arsenotherapy, those with *Streptobacillus moniliformis* are quite resistant, and as a consequence penicillin was tried successfully (1944). Penicillin in doses of 10,000 to 15,000 units administered intramuscularly every 3 hours, continued for 7 days is curative. Blood cultures were found to be negative and arthritic symptoms were improved and later disappeared. The patient has no relapse for a year.

The growth of streptobacillus from the patients blood was inhibited by 0.001 unit of penicillin per ccm in media in which it normally showed a luxuriant growth.

5. Other Spirochaetal Diseases

(1) Infectious Jaundice (Weil's Disease)

Aetiology.

Infectious Jaundice is an acute infectious disease characterized by a sudden onset with high fever, headache, vomiting, and jaundice which usually appears about the 3rd or 4th day. There is bile stained albuminuria; haemorrhages especially epistaxis are common and the liver and sometimes the spleen are enlarged. The disease is produced by *L. icterohaemorrhagica and L. canicola.*

The disease is of world-wide distribution and is common in Japan and Egypt and is also endemic along the Mediterranean coast. In recent years cases have been reported from many countries of Europe and America. The disease is also reported to be frequent in Burma and the East Indies. Although the disease has been presumed to exist in India for a long time, the first case was reported by Chopra and Das Gupta in 1937. Since then, a number of cases have been reported. Ordinarily only sporadic cases occur, but localised outbreaks are seen. In cold countries it is common in the warmer months.

Mode of transmision.

Human infection results from ingestion of food or water contaminated with the urine of infected rats, the leptospira gaining entrance through the skin and mucous membranes, the conjunctivae, mouth and intestines. Bites by infected rats may convey the disease. Bathing in infected water is a common mode of spread. Mice, rabbits and other rodents may be infected. Infections occur from handling soil and sewage and infected laboratory rats; attendants should wear rubber gloves when handling these animals.

Leptospira icterohaemorrhagica.

The *Leptospira icterohaemorrhagiae* was first discovered in 1915. It is a slender organism varying in size from 6 to 14 μ by 0.25 μ The spirals are closely placed together, giving the appearance of a series of small dots. It is actively motile, and may be demonstrated by the dark ground illumination, and by Giemsa's stain, or by the silver impregnation method or in Indian ink preparations. The organism is frequently found in the blood during the first 3 or 4 days of the disease. It is found in the liver and kidneys of inoculated animals, and can be grown on special media containing blood serum.

Symptomatology.

After an incubation period of from 6 to 12 days the disease sets in abruptly with fever, rigors, headache, muscular pains and vomiting, which may last for a few days and then pass off. Often it lasts for two to four weeks. The fever is of irregular type and runs between 102 to 104°F for the first 3 or 4 days when it begins to fall by lysis, occasionally by crisis, about the fifth day. In severe cases the temperature may not decline until about the 10th day. After a few days of apyrexia or moderate temperature, there is a tendency for a second rise towards the end of the 2nd week, which continues for about another week, when a slow convalescence sets in, in favourable cases. Jaundice usually appears about the second or third day with marked tenderness of the liver and gall bladder, and sometimes slight enlargement of the spleen. Pruritis is rare. The urine is scanty, high coloured and contains albumin and large amount of bile pigment. Early in the 2nd weeks, bile stained urine of low specific gravity is usually secreted in large amounts. Sleeplessness and nocturnal delerium occur, and in unfavourable cases the condition may resemble the typhoid state. Pains in the nape of the neck and calf muscles are common. In some outbreaks, haemorrhages, particularly expistaxes and malaena, are common; hæmaturia is rare. Petechial rash is common in some outbreaks and a measly or urticarial eruption may appear on fifth to twelfth day.

Blood pressure.

There is leucocytosis from 10,000 to 20,000; red cells and haemoglobin are both decreased and anæmia may last for weeks. Pulse is rapid at first, but with the onset of jaundice is slowed; blood pressure is low. Occasionally irido-cyclitis may occur.

The Vandenberg reaction is directly positive, the blood urea may be raised 50 to 397 mgm and death from uraemia may occur. Leptospirae which are found in the blood during the early days, soon disappear and after 7 to 19 days may appear in the urine, where they may persist for as long as 6 weeks.

Mortality.

The mortality figures vary in different countries from 4 to 32 per cent in Europe, to 48 per cent in Japan. In India the mortality is high like Japan.

Diagnosis.

Diagnosis.—Accurate diagnosis is only possible in the laboratory and depends on (1) discovery of leptospirae in fresh blood; (2) by culture from blood; (3) by inoculation of blood or urine into guinea pigs intraperitoneally and making smears from liver and spleen at the height of fever; (4) detection of organism in urine; (5) serological examination. The disease must be distinguished from bilious remittent fever, syphilis of the liver, yellow fever, liver abscess, relapsing fever and black water fever.

Prophylaxis.

Prophylactic measures include extermination of rats; prevention of contamination of food and water by discharges from rats; avoidance of bathing in infected pools, canals, and sluggish rivers. Sewer workers should be given protective inoculation. Laboratory workers handling rats and mice should wear rubber gloves. Prophylactic vaccination with killed cultures of the organism or a phenolized emulsion of the liver containing the leptospirae, have been tried with favourable results. More recently living avirulent cultures have been used for this purpose, but these are not without danger.

Treatment.

Treatment.—The treatment is largely symptomatic on the same lines as yellow fever. The patient should be given a liquid diet, and the bowels should be kept open regularly. Mild cases require little treatment but in severe cases there may be severe toxaemia, vomiting and nephritic symptoms which require energetic treatment. Intravenous injections of 500 to 1,000 ccm of a glucose solution, should be given. A polyvalent antiserum is of value in severe cases, and injections of 20 ccm should be given intravenously two or three times a day, for at least three to four days. The serum is most beneficial if given early in the disease, and its value is greatly reduced if it is given after the jaundice has appeared.. Convalescent serum, given in doses of 30 cc for three or more days, has been reported to have given good results.

If vomiting is severe, nutriment may be given in the form of rectal enemas, or substantial quantities of glucose may be given parenterally. Calcium injection are claimed to be of some value.

It has recently been demonstrated experimentally that *L. icterohaemorrhagica* infections are sensitive to penicillin therapy, which should be continued for from 7 to 10 days; and total of 1,000,000 units being given.

(2) Japanese Seven Days Fever, (Nanukayami)

This disease is quite different from the Indian seven days fever which is a form of dengue. The Japanese disease is related more to Weils' disease and is caused by *Leptospira hebdomadis*. The infection of the field mice transmits infection to the soil with urine and man is infected through the skin by handling the soil or by swallowing food with infected hands. Bites of infected rats may convey this disease. The fever lasts for about seven days and runs the same course as mild Weil's disease. It differs from dengue in that there is leucocytosis.

The spirochaetes occur in the blood in small numbers and are difficult to detect except by expert laboratory investigation as in Weils' disease.

Short spirochaetal fever occurs in Dutch East Indies lasting for 2 to 7 days. Several distinct strains of leptospira have been isolated. Rats and less commonly cats are the reservoirs of infection.

Megaw has pointed out that a number of these leptospira fevers occur in different parts of the world which have certain features in common. They are usually of short duration and have a tendency to relapse; they all produce jaundice, albuminuria and leucocytosis. They are liable to be confused with the dengue group. A severe type of non-spirochætal febrile jaundice, probably due to a virus, occurs which is difficult to differentiate from Weils' disease. The troublesome cases of jaundice occuring after inoculation against yellow fever combined with immune serum were suspected to be due to this virus originating in the blood used in preparing the vaccine. It is very difficult to sterilize any serum against viruses by filtration.

Vincent's angina is generally regarded as being due to a spirochæte and the *Bacillus fusiformis*. Injections of arsphenamine are very useful in this condition. The influence of arsenicals on the saprophytic spirochætes of the mouth is very doubtful and the role of spirochætes in the production of bronchitis is highly uncertain. In cases of pulmonary gangrene associated with spirochætes in the lungs, excellent results have been obtained by injection of arsphenamine.

6. Arsenical Compounds

Although arsenic was first used in medicine in England in the early part of the 17th century, its authentic history does not begin till Sir Thomas Fowler introduced Fowler's solution in 1786. Its employment in the treatment of protozoal disease was first suggested by the famous explorer, Dr. Livingston who recommended its use in the disease caused by the tsetse fly in animals.

(1) Pharmacological Action

Arsenic is toxic to all animals which possess a central nervous system and to most of the higher plants, but not to all lower organisms. Its antiseptic action is feeble and it is not classified as a protoplasmic poison. Arsenious acid is only one-tenth as strong an antiseptic as perchloride of mercury. Compounds of arsenic have no effect on the activities of such ferments as pepsin. Some of the pathogenic protozoa, *e.g.*, trypanosomes, show a most extraordinary susceptibility to arsenic and are destroyed in 1 in 200,000 dilution in the blood, while free-living protozoa may survive in 1 in 5,000 solution.

Local action.

Arsenicals do not coagulate proteins nor change them in any way and, therefore, they produce very little irritation of raw surfaces and mucous membranes, but the cells die slowly after prolonged contact with them. They are for this reason used for destruction of exposed dental nerves and epithelial growths of the skin. When applied to mucous membranes and denuded surfaces, they produce much pain and deep destruction of the tissues. Arsenical dermatitis occurs among workers who handle it.

In man, arsenic has a beneficial effect on the nutrition of the skin; the subcutaneous fat is increased and the complexion is improved; it renders the coat of domestic animals such as the horse thicker and more glossy. In veterinary medicine it is commonly used for this purpose and solutions of arsenic are used as 'dip' to rid animals of ticks, etc. Large

doses cause eruptions and keratosis, but this is not due to vaso-dilator effects nor is there evidence of any action on the nerves. Arsenic compounds undoubtedly, during their excretion, have a marked action on the intestinal epithelium and it is probable that they also exert a specific action on the epithelial cells of the skin. The arsenicals also produce a brown colouration of the skin of the face and other parts which is known as 'melanosis'.

Alimentary canal.

Small doses such as one-sixtieth to one-fiftieth of a grain of the oxide are said to increase the appetite and aid digestion; larger doses irritate the whole of the gastro-intestinal tract producing swelling and congestion of the mucous membrane. Capillary paralysis is produced causing much exudation into the connective tissue; the epithelium is at first raised and can be easily rubbed off; then it is cast off as shreds or false membrane. The exudation poured into the lumen of the gut causes distension, increased peristalsis and profuse rice water stools, collapse and death may follow.

Circulation.

Circulation. Very small doses have no effect on the isolated frog's heart, large doses depress it. The mammalian heart is little affected but large doses intravenously produce a fall of blood pressure due to paralysis and dilatation of the capillaries. The vessels of the splanchnic area seem to be more susceptible to the action of arsenic than those of the rest of the body. Their dilatation leads to congestion of the bowels, fall of blood pressure and collapse. It has been experimentally shown that the capillaries are injured and become more permeable after arsenic poisoning. Intravenous injections of large quantities of saline cause œdema in animals to whom toxic doses of arsenic compounds have been given, while no such effects are produced in normal animals.

Blood.

Blood. The action on the blood is still obscure though it is largely prescribed in anæmia. Arsenic probably produces increased vascularity and stimulates the bone-marrow to increased formation of erythrocytes and hæmoglobin when anæmia exists. With ordinary therapeutic doses however, no changes either in the erythrocytes or the hæmoglobin content have been observed. In toxic doses there is extensive degeneration of the cells of the bone-marrow and anæmia is produced. In shed blood small quantities of arsenites protect the red cells from various hæmolytic agents, and this may partly explain its beneficial action in diseases like pernicious anæmia.

Respiration.

Respiration. Small amounts have a distinct stimulating effect on the respiratory centre; large amounts depress it. Small doses are said to depress the peripheral terminations of the vagi in the lungs; hence it is used in asthma.

Central nervous system.

Central nervous system. In the frog a descending paralysis is produced, the animal first losing its spontaneous movements and then its reflexes; the terminations of the motor nerves become affected later on. In chronic poisoning, peripheral neuritis and characteristic lesions in the nerve trunks occur in man and animals and in severe cases the spinal cord may be secondarily involved.

Metabolism.

Metabolism. With small doses there is increased assimilation and decreased katabolism which give rise to improved digestion and increase in the body weight; metabolism of carbohydrates is slowed and fat is deposited. If large doses are given it causes increased breakdown of tissues as shown by augmented excretion of nitrogen, sulphates and phosphates in the urine. The glycogen in the tissues is decreased, lactic acid is markedly increased. Small necrotic areas are found in the liver and jaundice may occur. Excessive doses lead to loss of weight, fatty degeneration of organs and destruction of blood cells.

Fate in the body.

Fate in the body. Arsenic is absorbed readily even from the unbroken skin and mucous membranes. Poisoning may therefore result from the external use of arsenical cosmetic preparations. After hypodermic injection of large doses, diarrhœa may start within an hour.

Normal occurrance of arsenic in the body.

Normal occurrence of arsenic in the body. Arsenic is a normal constituent of the human tissues; but it plays no part in their functions. In man it occurs especially in the thyroid gland (0.16 mgm), in the thymus, brain, skin and hair; in the blood it has been estimated to be present to the extent of 0.3 mgm per 100 ccm of a dried specimen. Arsenic to the extent of 0.3 mgm has been found in 100 gm of dried normal urine. It is found in milk after administration for medicinal purposes and through the milk it passes from the mother to the infant. Large amounts of arsenic are found in the lungs. The arsenic occurring in the tissues normally is introduced with foods such as milk, eggs, fish, vegetable, wines, and also in drinking-water. According to the Royal Commission of 1903 the quantity of arsenic in the food should not exceed 1/100 gr per pound. From the medico-legal point of view the quantity of arsenic normally present in the intestinal canal never exceeds 1/10 mgm; larger quantities indicate poisoning or administration in the form of medicine.

Excretion. Arsenic disappears rapidly from the blood when injected and is fixed in certain tissues. It is chiefly found in the liver, kidneys, walls of the stomach and intestines, in the spleen and lungs. Much smaller quantities are found in the muscle and nerve tissue. With oral administration the main part leaves by the fæces; with hypodermic injection, by the urine. After a single dose excretion of arsenic goes on for ten days, and after repeated doses it may continue for months. It is found in tissues especially in the hair and epidermis for months after its disappearance in the urine and fæces. *Excretion.*

Tolerance. If an animal is given daily doses of arsenic in gradually increasing quantities, after some months the usual lethal dose may be given without any symptoms of poisoning being produced. A similar tolerance can be established in man. The inhabitants of Syria consume arsenic in large quantities, as a general stimulant and to improve their complexions. As much as 7 or 8 grains (0.5 gm) a day of the oxide are taken without apparent harm. It is said to produce intoxicating effects, stimulation of sexual and respiratory functions and a feeling of strength and well-being. Withdrawal does not cause any abstinence symptoms. *Tolerance.*

The phenomenon of tolerance to arsenic has not been satisfactorily explained. Some consider that the tolerance is limited to the intestinal epithelium, which no longer undergoes inflammation and necrosis under arsenic and absorbs less of this drug. Considerable quantities of arsenic, however, occur in the urine of arsenic eaters and therefore absorption must occur. Another hypothesis is that an antitoxin is formed. Tolerance is acquired by the pathogenic protozoa, and arsenic-fast strains of trypanosomes have been experimentally produced.

(2) Arsenic Compounds

Inorganic Arsenic compounds. All inorganic compounds of arsenic are very poisonous but metallic arsenic is insoluble in water and passes through the alimentary canal, for the most part unchanged and without exerting much action. It is probable that the activity is due to a compound of oxygen and arsenic appearing as an ion. The most important oxides are As_2O_3, or As_4O_6 called arsenious anhydride commonly known as 'white arsenic' and As_2O_5 arsenic anhydride. *Arsenic and arsenious compounds.*

Arsenic and arsenious compounds. In *arsenic* compounds arsenic is pentavalent, and in *arsenious* it is trivalent. Trivalent arsenic (arsenites) is generally much more toxic for protozoa, bacteria and yeast cells than is pentavalent arsenic (arsenates). The pharmacological action of arsenic is said to be due to the negative ion AsO_3 of arsenious acid $H_2As\ O_3$, in which arsenic acts as a trivalent element. It is held that compounds in which arsenic is pentavalent, *i.e.*, derivatives of arsenic acid H_3AsO_4, are reduced to arsenious compounds in the tissues. The ratio of toxicity of arsenious to arsenic acids when given intravenously to rabbits is 6 to 1 and for the excised frog's heart 300 to 1.

Organic compounds of arsenic. The organic compounds in which arsenic is combined with carbon behave somewhat differently from the inorganic compounds. As long as they are present in an unchanged condition in the body, they do not exhibit the ordinary action of arsenic. During the process of oxidation and reduction in the body, ionic arsenic is gradually split off and the action of arsenic is produced. For this reason the action is delayed and is mild. Both trivalent and pentavalent arsenic can be readily introduced into a large variety of organic molecules. The decomposition of organic arsenicals is, as a rule, slow and passes through many intermediate stages of simpler organic molecules; a considerable portion is in all probability excreted from the body in organic form during this process. The organic compounds are much more toxic to pathogenic protozoa infesting the animal body and that is the reason why they are largely used in the treatment of diseases produced by these parasites. When these compounds are tested, *in vitro*, they produce little or no toxic effect on the parasites and they therefore require the co-operation of the tissues of the host to become effective.

The pulmonary pressure is enormously increased after intravenous injection of arsphenamine probably due to the obstruction of the pulmonary circulation caused by the alkalinity of the solution and possibly also to the presence of an embolic precipitate formed in the vessels. The specific action of the drug on the musculature of the arterioles may be another factor concerned. Injections of salvarsan and neo-salvarsan in patients lower the arterial blood pressure.

Absorption and fate of the organic arsenic compounds. The rate of absorption varies with the modes of administration. After intramuscular injection in rabbits, of neoarsphenamine, 80 per cent of the arsenic had been absorbed during the first week and 90 per cent by the end of the second, while only about 5 per cent remained in the tissues at the end of six weeks. Arsphenamine or neoarsphenamine when given intravenously leaves the blood *Absorption and fate of the organic arsenic compounds.*

stream rapidly, at least three-fourths disappearing in a few minutes. Observations in animals show that after a single injection of one of the organic arsenicals, the arsenic is found abundantly in the organs whose capillaries would mechanically absorb the flocculates, the liver, spleen and the lungs, the size of the former making it the principal storehouse of arsenic. The liver gets rid of arsenic by excreting it in the bile, where its concentration is higher than in the blood; of all the tissues the bones retain arsenic the longest. The brain, spinal cord, the nerves and the cerebro-spinal fluid contain traces.

Excretion of organic arsenicals.

Excretion of organic arsenicals. After a single dose of pentavalent compounds, the arsenic is excreted promptly, and mostly in the urine. With continued administration, the excretion is prolonged, the greater part leaving within 3 days, but traces continue for 3 weeks; the effect is cumulative. A small part is excreted by the fæces. The excretion of atoxyl commences early about 85 per cent of the dose injected is excreted in the urine within 24 hours. The reason for this rapid excretion is that pentavalent arsenic is not fixed in the body cells.

Trivalent compounds.

Trivalent compounds. Salvarsan is excreted very slowly, the maximum amount of arsenic occurring in the urine being about 1.0 per cent in 24 hours and a somewhat larger amount occurs in the fæces, the total amounting to about 3 per cent. The drug is excreted more slowly after intramuscular than after intravenous injections. About 80 per cent leaves the blood in five minutes after an intravenous injection, but small quantities circulate in the blood unchanged for 12 hours, and traces of arsenic can be detected in the blood for 12 days. The greater portion of the drug is fixed in the liver where it forms a depot from which it is released slowly. Neo-salvarsan disappears from the blood slightly more rapidly than salvarsan and is said to be excreted more rapidly.

During their excretion inorganic arsenic compounds set up cloudy swelling of the epithelial cells, fatty degeneration and hyperaemia of the sub-mucosa of the gastro-intestinal tract; the kidneys are affected in the same way. Organic arsenicals produce the same changes though not to such a marked extent. Arsenic is also eliminated by the skin; it is known to have a marked affinity for the epithelial cells and it is probable that the production of exfoliative dermatitis can be accounted for in this way and the presence of peripheral neuritis may further facilitate its production. In cases of organic arsenicals also, traces of arsenic are excreted in the saliva and milk.

Classes of organic arsenic compounds. The starting point of all these are two acids, known as arsinic acid (trivalent) and arsonic acid (pentavalent).

Aliphatic series.

(*A*) *Aliphatic* (*or fatty*) *series.* The effects of cacodylic acid are essentially those of the inorganic arsenic, to which it is partly reduced in the body. Sodium cacodylate is probably such a stable compound that it is broken down in the tissues to a very small extent. Very large doses can be given without producing any therapeutic or toxic effect; it is excreted in the urine mostly unchanged but small quantities may be oxidised.

Aromatic series.

(*B*) *Aromatic* (*Benzol*) *series.* The development of this series of compounds is due to the efforts of Ehrlich to obtain drugs which would destroy the organisms producing disease in the body. The starting point of Ehrlich's investigation was atoxyl which is the sodium salt of p-aminophenyl arsonic acid known as arsanilic acid. By multiplicity of changes, synthesis and experiments on thousands of infected animals, he endeavoured to find which atoms or groups of atoms and what grouping in the molecule increased the affinity for the parasites and reduced the toxicity to the cells of the host most. Substitutions in the NH_2 group may cause profound differences in the effects of the compounds—sometimes an increase and sometimes a decrease in the therapeutic effect. If acetamide is introduced into the NH_2 group, tryparsamide is formed which is ten times less toxic than arsanilic acid. Ehrlich, from his experiments, concluded that these pentavalent arsenic compounds such as atoxyl and its derivatives, were inactive in themselves and only acquired activity when they were changed into trivalent arsenic. This led him to seek for organic compounds in which arsenic was present in trivalent form. Many of these compounds were prepared and eventually salvarsan and neosalvarsan were discovered. During recent years powerful pentavalent compounds have also been prepared.

The aromatic compounds of arsenic may be divided into:—

Pentavalent compounds.

(*a*) Those in which arsenic is pentavalent. *Atoxyl* is the monosodium salt of p-aminophenyl arsonic acid or p-arsanilic acid. It is also known as *arsamin* or *soamin*. *Arsacetin* or sodium acetyl-p-aminophenyl arsonate is tolerated in much larger doses than atoxyl. *Carbarsone* or 4-carbamino-phenyl arsonic acid was first prepared by Ehrlich in 1909; it has been recently used in the treatment of amœbic dysentery. *Tryparsamide*

(sodium-n-phenyl-glycinamide-p-arsonate) was discovered at the Rockefeller Institute, NewYork. *Stovarsol* (sodium-3-acetylamino-4-oxyphenyl arsonate). *Acetylarsan* (a simple derivative of stovarsol), *Treparsol* (3-formylamino-4-hydroxyphenyl arsonic acid) are some of compounds used.

Trivalent compounds.

(*b*) *Those in which arsenic is trivalent. Arseno-benzene derivatives*:—These compounds have a di-benzol or arsenobenzene nucleus coupled together by two trivalent atoms of arsenic. *Salvarsan* is No. 606 of Ehrlich's series (dihydrochloride of dioxy-diamino-arseno-benzene) is also known as *arsphenamine, kharsivan, arsenobenzol, arsenobillon,* etc.

Neo-salvarsan (No.914 of Ehrlich's series) is a condensation product of salvarsan. It is known as *neokharsivan, neoarsphenamine, novarsenobenzol* and *novarsenobillon. Silver sodium salvarsan* and *silver neo-salvarsan* have a high chemotherapeutic index.

Sulpharsenol or sulpharsphenamine is disodium dihydroxy-diamino-arsenobenzene-n-dimethylene-sulphonate. It is also known as *sulpharsenobenzene, kharsulphan, myosalvarsan.*

Oxophenarsine Hydrochloride (Mapharside). A more recent preparation (3-amino-4-hydroxyphenyl arsine oxide hydrochloride) is known as mapharside. It was accepted by the U.S.A. Council on Pharmacy and Chemistry for inclusion in New and Non-official remedies in 1943 and has more recently been included in the U.S. Pharmecopiaea.

(3) Modes of Administration

Local application. One in 1,000 solution of salvarsan and neo-salvarsan exert spirochæticidal effects on *T. pallidum* and *T. pertenue* in chancres, mucous patches and other syphilitic and frambœsial ulcers. Under the microscope, 1 in 1,000 solution destroys the motility of the spirochætes immediately.

Oral and rectal administration. Arsphenamine is absorbed when given by the mouth but produces symptoms of gastro-intestinal irritation. Its therapeutic effect is feeble. Arsenical compounds are given by the mouth in treatment of amœbic dysentery and pentavalent compounds such as carbarsone, stovarsol and treparsol are now sold in tablet form. The rectal method has been tried with salvarsan but is even inferior to the oral route.

Subcutaneous and intramuscular injection. Subcutaneous and intramuscular injections have been tried in aqueous solution, olive oil or liquid paraffin but salvarsan tends to deposit locally and gives rise to pain and swelling even when the solutions are carefully neutralised or mixed with olive oil before injection. The rate of absorption from the muscles depends primarily on the degree of injury to the tissues; neo-salvarsan, which is neutral in reaction, produces less irritation than salvarsan, which is acid, and is therefore absorbed 5 to 6 times more quickly.

The absorption of sulpharsphenamine is considerably more rapid. It is the only one of the trivalent arsenicals which can be safely given by this route and produces the therapeutic effects desired. The advantages of the subcutaneous and intramuscular routes are that acute toxic reactions are much less common though they are not altogether eliminated; the disadvantages are that local reactions are produced. The pentavalent compounds such as atoxyl, tryparsamide, stovarsol, as a group produce less local reactions than trivalent compounds.

Intraspinal, intraperitoneal and intrapleural methods. Even when well diluted with spinal fluid, the arsenical compounds given in the spinal canal produce considerable irritation of the cord causing severe shooting pains; the respiratory and cardiac centres are not affected. This form of medication is as a rule not employed. As intraperitoneal and intrapleural injections produce irritation these are not recommended.

Intravenous route. The intravenous method is the one usually adopted. The disadvantage of the intravenous method is that the drug is rapidly eliminated and consequently the period of spirochæticidal activity is short. Besides, the technique is more difficult and the incidence of acute toxic reactions is higher, due to factors inherent in the drug itself or in its solvents. The main advantage is the freedom from pain and disability when intravenous injections are properly given. The intravenous route is preferred in acute early stages of syphilis for rapid destruction of spirochætes in lymph, blood, and tissues; in later stages however when most of the organisms are fixed in the tissues, the intramuscular and subcutaneous routes are preferred by some, because the absorption and excretion rates are reduced and spirochæticidal effects are enhanced. Some give intravenous and intramuscular injections alternately. After intravenous injections the reticulo-endothelial cells of the spleen, liver and lymph glands become swollen, and granules of arsenic can actually be demonstrated in them. After very large doses of neo-salvarsan the granules can be demonstrated in the capillary endothelium, the intestine and the salivary glands as well.

The elaborate technique of prepartion, neutralisation and injection of salvarsan is no longer necessary with neo-salvarsan and other allied compounds, as they have a neutral reaction when dissolved in distilled water. The temperature of the solution should not exceed 22°C or 71°F. Slight febrile reaction generally occur after the first injection; if this reaction reappears in the course of the treatment (*i.e.*, on subsequent injections) it is a sign of intolerance. The rate of injection of these compounds is of importance. A concentrated solution injected slowly is as well borne as a dilute solution injected rapidly; but if injected rapidly they both produce a higher incidence of toxic reactions. With solutions of neo-salvarsan, sulfarsenol and tryparsamide the importance of rapid injections is not so great as with those of salvarsan, as the former being neutral in reaction do not produce intravascular reactions such as hæmolysis, agglutination and precipitation. The kidneys should be carefully watched and the urine tested for albumin before each injection. Sometimes the rubber tubing, through which solutions pass, may produce toxic symptoms due to the inferior quality of rubber.

A study of the various vehicles for the injection of the novarsenobenzol compounds has been made. In order to get the quickest and best results in the treatment of syphilis, the tendency is to give these preparations in doses as large as possible and to shorten the interval between courses. This unfortunately often produces toxic and sometimes fatal results. In order to avoid angioneurotic symptoms, dermatitis and other complications, a number of diluents other than saline were tried. Blood serum, glucose, saccharose, calcium chloride, egg albumin, and gum, have all been tested. Of all these, glucose appears to be the best as to some extent it prevents oxidation. Thirty ccm of a 30 per cent solution of glucose, given intravenously in conjunction with neo-arsphenamine is said to increase the efficacy of the latter.

(4) Organic Compounds of Arsenic

(i) Atoxyl

Synonyms:—Soamin, Sodium-p-arsanilate, Arsamin, Trypoxyl, Atoxylon.

It is a white odourless crystalline powder with a saline taste, soluble in 6 parts of water and 125 parts of alcohol. It contains 27.2 per cent of arsenic. Under the misleading name of atoxyl (for the substance is quite toxic), it was extensively used at one time in trypanosomiasis (sleeping sickness), syphilis and other protozoal diseases. A number of compounds similar to atoxyl have been tried in trypanosomiasis. *Soamin* is practically identical with

atoxyl, differing only in the amount of water of crystallisation. *Arsacetin* resembles atoxyl closely in its effects. Atoxyl is not given by the mouth but is usually given hypodermically or by intramuscular injections into the buttock in 10 per cent solution. The dose is 0.02 to 0.2 gm every other day. It is non-irritant, is absorbed rapidly and circulates in the blood longer than the arsenites. Atoxyl by solutions should always be freshly prepared and sterilised before use.

Atoxyl has no action on the trypanosomes *in vitro* and can only become effective when it undergoes certain changes in the body. Most of it passes through the body unchanged and the small portion which remains is changed into bodies which are capable of destroying the parasites. Thomas and Breinl (1905) found that atoxyl cures experimental trypanosomiasis in mice; Koch (1907) reported beneficial results in similar infections. Favourable results were also obtained in chicken spirillosis. Vœgtlin and Smith (1920) showed that the maximum tolerated dose (M.T.D.) of anhydrous atoxyl is 239 mgm, the minimum lethal dose (M.L.D.) 358 mgm and the minimum effective dose (M.E.D.) in experimental trypanosomiasis is 89.6 mgm per kilo body weight

Therapeutic uses. Atoxyl was introduced in the treatment of trypanosomiasis many years ago and is still used in animals. It acts on the parasites in the blood, but has less effect on those which have infected the lymph glands. On administration of this drug the parasites disappear from the blood, but the supply is being constantly renewed from foci in the lymphatic glands and in the nervous system.

Toxic effects. Toxic effects produced with doses exceeding 0.5 gm resemble the acute symptoms occurring in ordinary arsenic poisoning; these are dryness of the throat, headache, fever, colic, vomiting and diarrhœa, nephritis and paralysis of the lower limbs. The most serious effects are disturbances of vision, and these as a rule only occur when large doses of the drug are given at short intervals. They begin with scintillation, cloudiness, dimness and contraction of the field of vision, especially on the nasal side, which may lead to total and permanent blindness. Loss of vision can be easily produced in animals and is due to degeneration of the ganglion cells of the retina and later, of the fibres of the optic nerve. Though the retina is particularly susceptible, the cerebral cells are not immune and are also injured. Ophthalmoscopic examination shows at first no changes save that the retinal arteries may be narrowed and the veins somewhat hyperæmic; later there is complete optic atrophy. The most important factor in the production of optic lesions is the presence of the amino group in the para position to the arsenic. *Toxic effects.*

(ii) Tryparsamide

Synonyms:—Moranyl, Sodium-n-phenyl-glycinamide-p-arsonate.

This compound was synthesized and developed in the Rockefeller Institute. It is a white, odourless, crystalline powder containing not less than 25.1 and not more than 25.5 per cent of arsenic. It is very soluble in water forming a neutral solution which is comparatively stable, so that a 10 per cent solution can be boiled without decomposition. Its toxicity is very low and it is only slightly irritant and can be given intramuscularly, intraperitoneally (in animals) and intravenously.

The MLD for monkeys is 1.25 to 1.5 gm intravenously; slightly larger doses are required by the intramuscular route. Symptoms of intoxication are nervous and nutritional disturbances such as occur with all pentavalent compounds. There are tremors. inco-ordination of movements, clonic spasms, weakness and prostration; the appetite is lost and animals are emaciated and occasionally suffer from diarrhœa. The pathological changes consist of vascular dilatation, congestion, scattered petechial hæmorrhages and degenerative changes in internal organs. The minimum therapeutic dose for mice infected with *T. brucei* was found to be 0.2 gm per kilo when given subcutaneously and intravenously, and 0.275 gm when given intraperitoneally within 24 hours after infection. The chemotherapeutic index for mice was 1:8 while for rats infected with *T. evansi* it was 1:3. Its trypanocidal activity *in vitro* is feeble as the organisms remain motile in 1 in 100 solution. In mice injected with *T. brucei* the trypanosomes rapidly disappear, the ratio between curative and lethal dose being 1 to 10 as compared with 1 to 4 of atoxyl.

Dosage and method of administration.

Dosage and method of administration. In adults the dose is 2 to 3 gm and in children 0.5 to 1 gm according to age. The drug has been tried in sleeping sickness in doses of 2 gm intravenously (0.112 gm per kilo body weight). Solutions should be freshly prepared, 2 to 3 gm being dissolved in 10 ccm of cold sterile water. Such solutions should be clear but it is advisable to filter them through sterile filter papers, any turbidity necessitating the rejection of the solution. Boiling should be avoided as it may produce decomposition and toxic compounds. The drug is well tolerated intramuscularly in 20 per cent solution; 30 per cent solutions produce more discomfort and may lead to abscess formation. It may be given by this route but usually the intravenous route is preferred. Subcutaneous injections are not recommended as they produce irritation and suppuration. Ten weekly injections of 40 mgm per kilo body weight in a 10 per cent solution are considered safe. The toxic effects of this drug are confined to doses close to the MLD and the recovery of animals from sublethal intoxications is remarkably rapid and complete. This makes possible the repeated administration of very large doses of the drug at comparatively short intervals without incurring the danger of cumulative toxic action. A high degree of tolerance develops.

Toxic effects.

Toxic effects. Untoward effects are not common. The most serious such as amblyopia, nitritoid reactions and exfoliative dermatitis have not been noticed and jaundice is a rare complication. As a rule 80 to 90 per cent of the drug is excreted during the first 24 hours after injection, but in certain individuals it is excreted slowly and they are more susceptible to the cumulative effects of the drug.

The untoward effects generally appear after the fifth or sixth injection, the symptoms consisting of a sensation of dazzling; on examination the fundus is found to be hyperæmic; the visual fields are contracted on the nasal side. In severe cases contraction goes on till complete blindness results. Involvement of the optic nerve is by no means rare in neurosyphilis. All such cases should therefore be thoroughly examined by an ophthalmologist before treatment with tryparsamide. The visual fields, including the fields for colour vision, should be mapped out before its use, and should be checked several times during the course of treatment. Occasionally vomiting, slowing of the pulse and loss of consciousness may occur immediately after an injection, similar to those seen with arsenobenzene. Remote effects are shedding of the nails, albuminuria and dermatitis. Visual disturbances are seen with larger doses. If these occur a suitable interval between injections prevents them. Doses of 3.0 gm and over should not be given oftener than once a week. If amblyopia develops during the treatment of trypanosomiasis, tartar emetic is substituted for 3 weeks. Sodium thiosulphate injections appear to be ineffective in preventing amblyopia.

Therapeutic uses. Syphilis. Experiments on rabbits showed that this drug possesses the power of tissue penetrability to a marked degree; extensive cutaneous and subcutaneous lesions in these animals retrogressed and healed very quickly under its effect. Tryparsamide is not effective against primary and secondary syphilis, nor against the gummata or earlier cerebro-spinal manifestations. It appears to possess the power of penetrating into the tissues, especially nervous tissues, to a much greater extent than some of the other arsenicals. It has been tried in neuro-syphilis with good results.

The best results have been obtained in patients with early symptoms of dementia paralytica and nearly 50 per cent of cases are said to have shown varying degrees of symptomatic improvement. In tabetics, the drug is less satisfactory as also in advanced cases of dementia; in fact, in some of these

cases it may hasten the progress of disease. It is not advisable to use this drug in any other form of syphilis except that of the nervous system, and it is often used as a follow-up treatment after malaria therapy.

Tryparsamide is the drug of choice in later stages of human trypanosomiasis; in combination with Bayer 205, quite a large number of cases are cured (see trypanosomiasis). Tryparsamide is sold either as powder in 50 gm bottles or in ampoules containing 1, 2 and 3 gm.

(iii) Stovarsol (Acetarsone)

Synonyms:—Ehrlich 594, Fourneau 190, Acetarsone, Stovarsolan, Acetylamino-oxyphenyl-arsonic acid, Troposan. It contains 27.1 to 27.4 per cent of arsenic. It is absorbed rapidly, has a low toxicity and is excreted rapidly in the urine. Saulet (1927) found that stovarsol was lethal to *E. histolytica* culture within 24 hours in dilutions of 1 in 600. He noted that it was more active in the presence of liver extracts.

Syphilis. It was first introduced into medical practice in 1921-22 in the treatment of malaria and amœbic dysentery, as a prophylactic against syphilis, and for the treatment of spirochætal infections. Administration by the mouth produced healing of primary lesions in rabbits and monkeys.

In amœbic dysentery the drug has been tried in those cases who had resisted emetine treatment, in doses of 0.25 gm in pill form twice daily for a period of 10 days. Occurrence of a measles-like rash was not uncommonly met with and cases of acute exfoliative dermatitis have been recorded after administration of stovarsol. Knowles treated 32 patients, mostly chronic, in Calcutta; the ratio of probable cures to failures in these was 1: 1.1. The drug has also been tried in the treatment of malaria and relapsing fevers.

Toxic symptoms. Cases of poisoning with stovarsol, following its administration in dysentery in 2 gm doses daily, have been recorded. Fever was the first symptom followed 2 days later by diffuse erythema, dryness, pruritus and urticaria. Rashes appeared all over the body and on the legs and face; in severe cases it may pass on to exfoliative dermatitis. Oedema, jaundice, colic, diarrhœa, albuminuria, coryza, bronchitis, urticaria, ocular troubles, giddiness, collapse and glycosuria may occur. Acute nephritis with casts in the urine may occur after injection. The drug should not be given for more than 10 days at a time, the second course should be given after a suitable interval. *Toxic symptoms.*

(iv) Treparsol and Acetylarsan.

Synonyms:—Fourneau 257, Sodium-3-formylamino-4-oxyphenyl-1-arsonate, *Formyphenarsine.*

It is said to be suitable for administration by the mouth in 1.0 gm doses. In the intestines treparsol is split up into formic acid and meta-amino-para-oxyphenyl arsenic acid which is the active principle of stovarsol and is said to act directly on intestinal parasites, specially protozoa. Although courses extending over 4 weeks have been recommended it is not advisable to give it for more than 10 days at a time. Slight diarrhœa may occur and fatal cases have been recorded after prolonged use. Treparsol is excreted mostly in the urine.

Acetylarsan (hydroxy-acetyl-aminophenylarsinate of diethyl amine) can be given by intramuscular and subcutaneous injections, but headache, vomiting and diarrhœa are frequently produced; erythema, albuminuria, and jaundice may occur.

(v) Arsphenamine

Synonyms:—Salvarsan, Kharsivan, Dioxy-diamino-arseno-benzol dihydrochloride, Arsenobenzene, Arsenobenzol, Arsenobillon, Arsen-phenolamine, Amino-arsenophenol, Ehrlich-Hata 606.

Salvarsan was first prepared by Bertheim and tested by Ehrlich and Hata in 1910. The dose for women is 0.3 to 0.4 gm and for men 0.4 gm in 200 ccm of solution medium It is a yellow crystalline powder containing 31.5 per cent of arsenic; it is readily oxidised in the air and so is kept in vacuum tubes. Salvarsan though it does not kill spirochætes *in vitro* has a very pronounced effect on these organisms *in vivo*. It is fifty times less

toxic for experimental animals than mercury. The MLD for mice is 143 mgm per kilo and for rabbits 100 mgm when given intravenously. In rats showing a trypanosome count of 150,000 to 250,000 per ccm of blood, temporary sterilisation could be effected with doses of 6 mgm and permanent sterilisation with doses of 8 to 12 mgm per kilo. Mice infected with the spirochætes of relapsing fever were sterilised with 10.6 mgm per kilo and rabbits inoculated with syphilis were cured with 23.5 mgm per kilo given intravenously.

Salvarsan has the disadvantage of having an acid reaction in solution and therefore has to be neutralised at the time of use. For this reason it has been replaced by neosalvarsan. A solution which is not fully neutralised or one which is over-alkalinised is depressant for the heart. Neutralisation is best effected by adding to the salvarsan solution 15 per cent caustic soda solution to precipitate the base (0.28 ccm for 0.3 gm of the drug); dilute with a little 0.5 per cent saline and then add just enough caustic soda solution to dissolve (about 0.6 ccm in all); finally dilute to 300 ccm with 0.5 per cent saline solution.

(vi) Neo-arsphenamine

Synonyms:—Neo-salvarsan, Neo-kharsivan, Ehrlich 914. Novarsenobenzol, Novarsan, Novarsenobillon, Metarsenobenzol, Neo-arsenophenolamine, Sodium dioxy-diamino-arsenobenzene-methanesulphonate.

This is a condensation product of salvarsan. It differs from salvarsan in that it is not a combination of dioxy-diaminoarsenol-benzol with HCI but with sodium methane sulphonate. It consists chiefly of sodium 3, 3-diamino-4, 4-dihydroxy arsenobenzene-N-methanol sulphoxylate. It is a yellow powder, readily soluble in water, giving a neutral solution. It is a stable preparation and readily dissolves in water forming a neutral solution, it can therefore be injected intravenously in concentrated form. The dry powder must contain not less than 10 per cent or more than 21 per cent of arsenic. *In vitro* experiments show that during the first 10 hours even 1 in 100 concentrations have no effect on the spirochætes, but after 15 hours there is marked reduction in their number and eventually all are destroyed. The maximum dilution for killing them was 1 in 200,000 as compared with 1 in 2,500 for *Bact. coli.*

When injected intravenously into rats, neo-salvarsan is about two and a half times less toxic than salvarsan. The maximum tolerated dose of the American product in rats is 254 mgm per kilo body weight, in mice it is 250 mgm and in rabbits it is 200 mgm when given intravenously. The average minimum curative dose of neo-salvarsan for rats infected with *T. equiperdum* is 4 to 6 mgm intramuscularly and for rabbits inoculated with syphilis 30 to 40 mgm intravenously. The hydrogen-ion concentration of neo-salvarsan is 7 to 7.4 which is the same as that of the blood, while that of a properly alkalinised solution of salvarsan is more than 9. It therefore causes less biochemical disturbances in the blood and tissues.

Dosage.

Dosage. This should be regulated according to the general condition of the patient. In the male adult the starting dose of salvarsan is usually 0.2 gm which is increased by 0.1 gm at every successive injection till a maximum of 0.6 gm is attained. For females, the starting dose is 0.2 gm and a maximum of 0.4 gm, for children 0.1 to 0.3 gm; for infants 0.02 to 0.1 gm. The dose of neo-salvarsan should never be exceeded. The ordinary course is 6 to 8 intravenous injections given at intervals of 7 to 10 days. With very early cases, the first 2 or 3 injections may be given 3 or 4 days apart. The patient is allowed 6 to 8 weeks' rest and then another course is given and this in early cases may be repeated till 3 or possibly 4 courses have been given. The drug is combined with bismuth preparations.

The drug can be given by the intravenous or intramuscular route, the former being preferable. It should never be given subcutaneously. For gravity intravenous injections, 12.5 ccm of freshly double distilled pyrogen free water should be used for every 0.1 gm of the drug. The solution should be warmed to not more than 20 to 22° (68 to 71.68°) before injection. For intramuscular injections 0.15 gm is dissolved in 0.3 ccm of freshly distilled water; this will give an isotonic solution. Concentrated solutions 0.1 gm in 0.5 ccm of freshly distilled water may be given by a syringe. It is advisable to draw up equal amounts of blood in the syringe containing the solution and then reinject very slowly, the ampules containing the drug should be immersed in alcohol to detect a possible crack. The drug undergoes deterioration in air and becomes toxic. Any incompletely soluble product or those giving abnormal coloured solutions should be rejected. The drug in fresh solution has a canary yellow colour. The drug should be preferably kept in a cool dark room or in a refrigerator and should be not more than six months old. Neosalvarsan is sold in ampules containing 0.15, 0.3, 0.6, 0.75, 0.9 gm.

(vii) Oxophenarsine Hydrochloride (Mapharside)

Synonyms:—Mapharside (Parke, Davis & Co) Phenarsine Hydrochloride (Winthrop) Clorarsen (Squibb).

Mapharside is a tri-valent arsenical and contains 31 per cent arsenic. It is now officially designated in the U.S.P. as oxophenarsine hydrochloride. This preparation was introduced by Parke, Davis and Co in 1932 and has been in extensive clinical use ever since. In dry form mapharside is stable; in contact with traces of moisture and air its colour very slowly changes from white to gray or brown. As supplied for clinical use, mapharside is readily soluble in distilled water to form a solution suitable for intravenous injection.

In 1941, the therapeutic possibilities of buffered solution of oxophenarsine hydrochloride was presented in a report by the U.S. Council on Pharmacy and Chemistry based on a series of animal experiments and on a clinical study of 171 patients treated over a period of 2 years. It was concluded that the drug buffered with sodium citrate is "a safe and effective drug in the treatment of syphilis as judged by (1) rapid production of darkfield negativity of early lesions, (2) prompt healing of early lesions, (3) effectiveness in producing sero-negativity in early syphilis, (4) low percentage of relapses, (5) low incidence of abnormal spinal fluids in early syphilis, (6) absence of severe immediate reactions to its administration and relatively low number of reactions in general".

It is claimed for mapharside that it is the active derivative of arsphenamine formed in the body after this has been injected and, being already oxidized, it is stable. It is given in doses about one tenth of those used when giving neoarsphenamine.

Mapharside is a simpler compound than neoarsphenamine having one benzene ring.

It was one of Ehrlich's original compounds but he discarded it because he believed it to be too toxic. It is in fact less toxic than arsphenamine because it is more rapidly excreted; its excretion is completed in two days as compared with 5 to 7 days of arsphenamines.

The advantage of this drug is that its 60 mgm equal 600 mgm of neo-arsphenamine. The arsenic content of the blood from the beginning of the treatment on the third day and upto fourth and sixth day is 20 to 33 microgrammes per 100 ccm, *i.e.*, 1 in 3 millions to 1 in 5 millions. Experiments on rabbits showed that the total curative dose of mapharside was constant irrespective of the period over which the treatment was given(10 seconds to 6 weeks). The minimal curative dose remained at 4 to 8 mgm per kilo body weight and for intravenous drip from 1 to 4 days the limits were 7-12 mgm per kilo. The maximum

tolerated dose increased as the duration of treatment increased and since the curative dose was constant the margin of safety increased with duration. Eagle & Hogan considered that any desired margin of safety could be achieved by prolonging the period of treatment, *i.e.*, from intensive course of 5 days which was dangerous to prolonged course of 18 months which was vexatious.

The single weekly dose of mapharside is 1 mgm per kilo of body-weight for adults. The usual single dose for average adults is 60 mgm (0.06 gm). Regardless of the treatment method used, the total curative dose of mapharside for adults with early syphilis ranges from 1,200 to 2,400 mgm. Clinical results with all theraputic routines now in use (five days to eighteen months) are about the same, provided the total dose of mapharside is at least 1,200 mgm.

Infants and children tolerate mapharside satisfactorily. Initial doses should be on the basis of 0.5 mgm per kilo of body-weight. The maximum dose of 1 mgm per kilo may be administered weekly during regular courses thereafter.

Mapharside is said to have the lowest record of toxicity of any trivalent arsenicals used in the therapy of syphilis. Serious nitritoid crisis has not been reported. Mild cases of dermatitis may occur, although the incapacitating exfoliative type is very rarely encountered. Reactions most frequently observed have been transitory nausea, vomiting, lacrimation and pruritus. Of these, the gastric symptoms are the most troublesome. The low toxicity and high clinical effectiveness was recognized by the National Research Council (U.S.A.) in 1941. They concluded that "the preferred arsenical in and out of hospital is mapharside. When mapharside is not available, Neoarsphenamine may be used."

Silver salvarsan or silverarsphenamine is a metallic arsenobezene compound. It is a brownish-black powder, readily soluble in water giving an alkaline solution. It contains not less than 19.0 per cent of arsenic and 12 to 14 per cent of silver. It is given intravenously in doses of 0.1 to 0.2 gm for women and 0.25 gm for men at weekly intervals.

Neo-silverarsphenamine or neo-silver salvarsan is a molecular compound of neo-salvarsan and silver and is a brown powder. Sodium silver neo-salvarsan is yet another preparation. Neo-silver salvarsan is said to be about half as toxic as silver salvarsan and slightly more toxic than neo-salvarsan; its maximum tolerated dose is 278 mgm per kilo of mice as compared with 167 mgm of silver salvarsan and 370 mgm of neo-salvarsan. These compounds have the same actions as the ordinary compounds.

(viii) Sulpharsphenamine

Synonyms:—Sulfarsenol, Sulpharsenobenzene, Myosalvarsan.

This was originally a French preparation. It is a fine yellowish powder which dissolves easily in water giving a neutral solution which can be given intravenously, subcutaneously or intramuscularly. It contains not less than 19 per cent of arsenic and 8.7 to 12.8 per cent of sulphur. Chemically it is dioxy-diamino-arsenobenzene-dimethylene sulphonate. Dose 0.12 to 0.18 gm for the first injection in 2 to 3 ccm of distilled water, gradually increased to 0.45 to 0.6 gm. For intramuscular or subcutaneous injections 0.1 gm of the drug is dissolved in 0.3 ccm of freshly distilled water, the total volume should not exceed, 1.0 to 2.0 ccm. There is less reaction if minimum quantity of diluent is used.

For intravenous injections 0.06 gm is dissolved in 6.0 ccm preferably 20 ccm or more of double distilled pyrogen free water. Harrison (1921) does not recommend the intravenous use of the drug as dermatitis and other untoward effects are more likely to follow by this route. The MLD (minimum lethal dose) of this drug in white rats ranges from 320 to 480 mgm and the MED (minimum effective dose) from 15.9 to 31.5 mgm per kilo when given intravenously. When given intramuscularly or subcutaneously, the MLD. is 400 to 700 mgm and the MED. is 15.6 to 34 mgm per kilo body weight. It is equal in efficiency to salvarsan or neo-salvarsan in the treatment of syphilis when given by the hypodermic or intramuscular route; it is therefore useful in children and for obese persons with veins difficult to find; such strong solutions as 20 to 30 per cent can be given without untoward symptoms. The drug is useful in patients who develop shock or nitritoid reactions. It is therapeutically just as effective and is specially well borne by children with congenital

syphilis. Dermatitis and jaundice may be produced by it, but acute symptoms such as nausea, vomiting and diarrhœa are less common; the incidence of general reactions is also lower. Harrison (1923) recommends it when myocarditis and aneurysm are present. Patients receiving sulpharsphenamine should be carefully watched as there is higher incidence of reactions than other arsenicals. Those met with are:—dermatitis, hæmorrhagic eruptions, meningo-vascular reactions, and aplastic anæmias. The drug is sold in ampules containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.9 gm.

(ix) Other Arsenical Compounds

Thiosarmine or disodium-dioxy-diamino-arsenobenzene-methylene sulphonate has been prepared in the Brahmachari Research Institute, Calcutta. It is very closely allied to other sulpharsenobenzene compounds such as sulfarsenol, kharsulphan, sulpharsphenamine, etc.

Thiosarmine is a light yellow powder readily soluble in water, almost neutral in reaction and having a hydrogen-ion-concentration of 7.2 to 7.4. It is fairly stable and does not change its colour, even on standing for 48 to 72 hours. When heated it decomposes without melting. The arsenic content varies from 19.5 to 25 per cent.

Toxicity. The toxicity of the compound is low.Given intravenously in white rats the maximum tolerated dose of thiosarmine was 300 mgm and the minimum lethal dose 490 mgm per kilo of body weight. The minimum lethal dose in the author's series of experiments, when given intravenously in a 2 per cent solution in white mice, was found to be between 425 to 450 mgm per kilo of body weight.

Thiosarmine is indicated in all stages of syphilis. The dosage recommended in syphilis is to begin with 0.15 gm increased by 0.15 gm in subsequent injections to a maximum single dose of 0.6 gm. Below 16 years ¾ full dose is given and children below that age should receive proportionate dosage. The drug should be dissolved in redistilled water, boiled and cooled. A 10 per cent solution is slowly injected intramuscularly in the gluteal region. It can also be given subcutaneously or by the intravenous route.

Bismarsen or bismuth arsphenamine sulphonate. It is one of the co-ordination compounds of arsphenamine, the bismuth being linked to the arseno group in the same way as silver, gold, etc., to their respective compounds. Chemically it is trisodium-3-amino-4-hydroxy-phenylarseno-n-methylene-sulphonate dibismuth. Bismarsen is a brownish-yellow amorphous powder freely soluble in cold water. The arsenic content of the preparation ranges from 12 to 15 per cent while bismuth occurs in proportion of 23 to 25 per cent. One advantage of the drug is that it can be given intramuscularly, and at the same time the combination of bismuth and arsphenamine may enhance the spirochæticidal activity to an extent greater than the summation of these two drugs separately. The drug is slower in action than the arsenicals and there is pain at the site of injection.

Dosage:—The initial dose is 0.1 gm and succeeding dose 0.2 gm. The drug is dissolved immediately before injection in 1 to 2 ccm of a sterile aqueous solution of 0.25 per cent, butyl sulphate. Weekly doses are given at first, later on biweekly injections may be given.

Mode of action of organic arsenicals.

Mode of action of organic arsenicals. Ehrlich's original idea was that these drugs had a simple parasiticidal action and that certain chemical chains in the drugs had a selective chemical affinity for certain side chains in the protoplasm of the organisms and therefore the organisms were killed without harm to the host; this idea has now been given up. That organic arsenical compounds are converted into trivalent oxides before parasiticidal action is brought about, has been experimentally shown. Several drugs have no action upon trypanosomes *in vitro,* but have a considerable trypanocidal action *in vivo* and it has been proved by experiments that these drugs are in many cases changed in the body from relatively inactive to more active forms. Hata working in Ehrlich's laboratory showed that arseno-benzene does not kill spirochætes *in vitro.* In the Hygienic Laboratory, U.S.A. Public Health Department, it was shown that a partial oxidation of arsenobenzene occurs. The compounds of R. As.=O type (arsenious acid, arsenoxide) in dilutions of 1 in 10,000 render trypanosomes non-infectious *in vitro* and kill the trypanosomes in rat's blood immediately after injection. The compounds of R.As.=As.R. (salvarsan) type in similar doses have little action on trypanosomes for the first hour and do not kill 90 per cent of the parasites after 2 or 3 hours. It seems that it is the persistent effect of minute quantities of the oxide, far below the concentration immediately lethal *in vitro,* maintained for sometime, which is responsible for cures.

A new conception of the arsphenamine treatment of syphilis is offered by Anwyl-Davis. While watching *S. pallidum* for days by the dark ground method he found that when bathed in salvarsanized serum they gradually lose their normal appearance, become ghost-like, fragmented and paralysed and in about five days apparently die. About 5 or 6 hours later they recover their normal appearance, are revivified, regain their spiral and motility and become as normal and as active as controls that have not been 'doped.' This strongly suggests that arsphenamine should be injected at intervals of five days or less instead of waiting for the customary week. The organisms should be attacked with fresh drug when their vitality is lowest so that they may not multiply or develop resistance.

These drugs are examples of compounds whose therapeutic efficiency is due to changes which take place in them in the body. Their therapeutic efficiency is estimated by determining the ratio between the sterilising dose and the tolerated dose though this is liable to error on account of the resistance of different species of trypanosomes and also of the animals. Voegtlin and his associates (1923) have advanced the theory that arsenic in the form of trivalent oxides (R.A.s.=O) is the active form and is a specific poison for the sulph-hydryl SH groups of compounds like glutathione and possibly other SH compounds present in the protoplasm of the parasites. The glutathione group is concerned in the oxidation and reduction processes in the living cells. Arsenic therefore in the form of an aromatic arsenious compound may be regarded as a specific poison which acts by combining with the SH compound in the protoplasm and its effects can be counteracted by introducing the SH compound artificially, to take the place of those present in the protoplasm. The organic compounds of arsenic are examples of drugs which are introduced into the body in a relatively inert form and are changed by the body tissues to therapeutically powerful compounds.

(5) Toxic Effects of Arsenic Compounds

Acute poisoning.

Inorganic arsenic, when taken by the mouth in large doses produces severe gastro-intestinal irritation, vomiting, painful profuse watery diarrhœa (rice water stools), suppression of urine, intense thirst, prostration and collapse; even when given hypodermically the drug produces its action on the epithelium of the gut, producing fatty degeneration, necrosis and ulceration. The symptoms usually appear within half to one hour after it is taken by the mouth. In fulminant cases, the accumulation of blood from the general circulation into the splanchnic area on account of capillary paralysis, may produce collapse and death without producing any of the usual symptoms. At the post mortem the stomach and intestines are found inflamed and there are patches of softened mucous membrane. If the patient survives long enough, fatty degeneration of the liver, kidney and heart is seen.

Fatal doses of white arsenic vary with the solubility of the preparation. Five to 50 mgm of the trioxide produce toxic symptoms, while 0.1 to 0.3 gm are fatal, but recovery may occur after much larger quantities.

Chronic poisoning.

Chronic poisoning. The symptoms which appear first are loss of appetite, nausea, vomiting, colic, and mild diarrhœa; swelling of joints, peripheral neuritis, weakness, tremors of muscles, ataxic gait, muscular atrophy, bronzing and patchy pigmentation of the skin, darting pains in the limbs and muscular paralysis may occur. Persistent capillary paralysis produces diarrhœa and œdema; sensation may be dulled and blindness may be produced. The endothelium of the capillaries, later the intestinal epithelium and finally the cells of such organs as the liver, kidneys, and the heart undergo fatty degeneration. Arsenic is quickly absorbed and is slowly excreted; it is therefore a cumulative poison. Pigmentation of the skin, dermatitis and peripheral neuritis commonly occur in chronic poisoning.

Treatment of arsenic poisoning.

Treatment of arsenic poisoning. Acute arsenic poisoning is usually fatal, and therefore treatment should be started as early as possible. Elimination of the drug by emetics, lavage with warm water or purgation should be tried at the first instance. Chemical antidotes are not of much value. Ferric hydroxide with

magnesium oxide was long considered the best antidote, the ferric hydrate and arsenic forming an insoluble compound. It is now known that the antidote is not effective to the same extent as was believed and that its administration only gives a slightly smaller percentage of fatalities. In any case, as this antidote is harmless, it may be given a trial provided that it is promptly followed by effective evacuation.

Chronic arsenic poisoning does not require prompt and energetic treatment. The administration of arsenic in any form should be stopped. Rest in hygienic surroundings, a milk diet and alkalies are indicated. Symptomatic measures against vomiting, diarrhœa, etc., and stimulants whenever necessary may be exhibited.

Toxicity of organic compounds. Organic arsenicals such as salvarsan and its substitutes are usually injected in doses as high as can be tolerated and therefore toxic effects are not uncommon. These are generally due to three causes; in order of importance they are—

1. *Pathological states and susceptibility of the patient.* Fear, nervousness, endocrine disturbances, vagotonia, pathological changes produced by syphilis, non-syphilitic diseases, mercury, etc., play an important part. Natural or acquired hypersensitiveness to arsenical compounds is another important factor.

2. *Errors in method of administration.* Failure to make proper solutions, *e.g.*, making them over-acid or over-alkaline, use of defective saline solutions, excessive dilutions, rapid intravenous injections, oxidation of the solution, emboli of the undissolved drug, cotton, air, etc., are factors of some importance.

3. *Properties of the drug itself.* The solutions may have toxic physical properties, agglutination of erythrocytes or precipitation of plasma may occur or hæmolysis may be produced. Oxidation of the drug may occur or toxic impurities may be present.

With all these possible causes of toxic reaction it is not surprising that various undesirable symptoms are produced. Some degree of systemic reaction occurs in 0.5 to 1.0 per cent of all injections, but although they are alarming they are not often dangerous. The fatality rate in a German clinic (1920) was reported to be 1 in 63,000 if the dosage was below 0.6 gm, and 1 in 3,000 if it was above. The danger can be reduced by using a reliable drug, proper technique and careful examination of the patient. The symptoms may be divided into three groups.

(i) Immediate, early and late reactions

A. *Immediate reactions.* In the great majority of cases after intravenous injection of neo-salvarsan and sulfarsenol by the technically correct method no untoward effects are noticed. The first effect mentioned by the patient is a peculiar odour, and metallic taste. This effect is probably due to the circulation of the drug in the mucous membrane of the nose and salivary glands and is noticed soon after the injection. Chewing of flavoured gum or cloves during injection does away with this. Not infrequently the patient experiences a feeling of fullness in the head, and slight flushing of the face which may only last a few minutes or less; these are due to the action of the drugs on the vasomotor system and are less marked with neo-salvarsan and sulfarsenol than with salvarsan. Slight perspiration and a feeling of faintness may be noticed. Nausea and vomiting sometimes occur half an hour after the injection in neurotic patients. Intramuscular and subcutaneous injections rarely produce nausea and vomiting and the other symptoms are as a rule absent. The more severe immediate reactions are as follows:—

Physico-haemoclastic reactions.

1. Acute physico-hæmoclastic reactions occur with salvarsan solutions, and these are due to errors in neutralisation and the symptoms are the result of widespread embolism and infarction, especially of the lungs, and cardiac dilatation; clumps of agglutinated erythrocytes are found in the heart and in the intima of blood vessels. During injection the expression of the patient's face becomes drawn, hiccough or short spasmodic cough may develop. Dyspnœa and suffocation ensue, the pulse becomes rapid, feeble and irregular; pain and constriction in the chest occur due to emboli in the lungs; convulsions may occur due to emboli in the brain; no demonstrable symptoms are produced by embolism of the liver, spleen, kidney and other organs. Broncho-pneumonia and pulmonary embolism may develop after an interval. The patient becomes anxious, expresses a fear of approaching death; coma may follow from cerebral anæmia. The patient may recover within ½ to 2 hours or die. The cause of this acute crisis is unknown though coagulation of the blood proteins has been suggested. There is no evidence to support this, but agglutination of red blood cells may occur *in vivo* as a result of injection of solutions of arsphenamine, and multiple emboli have been noted in the lungs.

The treatment in these cases, is to lay the patient flat on his back and to give 1 to 2 minims of 1 in 1,000 solution of adrenalin intravenously; for less severe reactions give 0.5 to 1.0 ccm intramuscularly. Atropine sulphate 1/50 grain may be given intramuscularly for stimulating the circulation. Intravascular agglutination can be prevented by injecting arsphenamine with a protective colloid such as gelatine.

Vasoparetic reactions.

2. Acute vasoparetic reactions or 'nitritoid crises,' so called by Milton (1912) because the symptoms and lesions resemble the inhalations of amyl-nitrite. These reactions are produced by intravenous injection of many substances and are common among individuals suffering from symptoms of adrenal insufficiency or thyroid disturbances. They have been attributed to the disturbance of the sympathetic system, but it is more likely that they are due to the toxicity of the drug or abnormal changes in the capillaries or a combination of the two. The symptoms occurring immediately after or during injection are flushing of the face, dilatation of the pupils, increased pulse rate and dimness of vision. There may be pain in the gums and teeth. In more severe cases, the lips and tongue become swollen, there is a feeling of constriction in the throat and upper part of the chest and dyspnœa; a tingling sensation in the extremities, congestion of the conjunctiva and lachrymation are always present. The patient may fall, if standing when the reaction comes on. In still more severe cases there may be urticaria, either limited to portions of the body or generalised over the limbs and trunk; profuse perspiration, vomiting, retching, loss of sphincter control and possibly loss of consciousness which may last several hours. These symptoms, though rare, occur in a certain percentage of cases after each injection. Silver salvarsan, unless injected very slowly, almost always causes vasomotor symptoms. The face becomes pale, the pulse feeble, and vomiting may ensue. In another type of reaction rigors and headache occur; it is more common after the first than subsequent injections. Occasionally vomiting is followed by severe diarrhœa a few hours after the injection. Herpes labialis may occur. In mild cases the patient may feel nothing but fright. The symptoms can all be explained by sudden dilatation of blood vessels especially the capillaries. Extreme cerebral and meningeal congestion may occur causing apoplexy. In mild cases there is only headache and vomiting and these soon pass off. Usually symptoms pass off in an hour but exceptionally may persist for several hours. Syncope may be a prelude to vomiting. The patient should have no solid food two hours before injection.

Although the symptoms described are very alarming they are rarely fatal. Cases of cardiac syphilis with aortic insufficiency and myocarditis are specially liable to develop alarming symptoms with sudden syncope after arsphenamine. In these cases cold sweats, gasping respiration, pallor, bradycardia and low blood pressure are associated with acute cardiac damage.

Various explanations have been suggested to account for the immediate vasomotor disturbances. As adrenalin relieves those symptoms, it was thought that insufficiency of the adrenal glands might be the cause. Another explanation is that a precipitate is formed. None of these hypotheses are supported by experimental data. The theory most favoured is that the symptoms resemble anaphylactic shock, and many of these certainly seem to be those due to liberation of histamine and histamine-like substances.

Treatment. The symptoms are prevented by slowness of intravenous injection and by injection of 0.5 to 1.0 ccm of adrenaline hydrochloride 1 : 1,000. A useful preventive of immediate reaction is to dissolve the dose in 2.0 ccm of water and the solution added to 8 ccm of 50 per cent solution of glucose. If symptoms appear the patient is made to lie flat and adrenalin 0.5 to 1 ccm of a 1 in 1,000 solution is given intramuscularly at once or it may be used as a prophylactic before the injection. Pituitrin may be given with adrenalin or by itself, atropine sulphate 1/50 gr subcutaneously relaxes the bronchi and relieves dyspnœa. In severe cases of coma give adrenalin intravenously. Ephedrine is said to be specially useful in preventing vasomotor paralysis. Fractional injection, *i.e.*, 1/10 of the dose an hour before the full dose, may be adopted as a prophylactic measure. In animals, injection of cæsinum eosinate prevents the symptom of anaphylactic shock and 15 ccm of a 6 per cent solution prevents shock in man; neoarsphenamine may be dissolved in this solution. Sometimes symptoms of increased cranial pressure or tension headache occur. In these cases magnesium sulphate should be given internally or by rectal enemata to produce dehydration. If symptoms of encephalitis appear the pressure may be relieved by drawing cerebro-spinal fluid, and adrenalin may be given. *Treatment.*

B. *Early reactions.* These generally start in 1 to 4 hours, but may occur within 24 hours after injection. There is fever, headache, protein or colloidal shock; accidents of injection such as phlebitis and thrombosis, also come under this heading. The kidneys may be involved and albumin and casts may be found in the urine. Temperature rarely exceeds 101°F. There is evidence that fever enhances the antisyphilitic effects of the remedy and some authorities give pyretic injections a few hours after the arsenicals. Gastro-intestinal symptoms occur if injections are given soon after a meal. These are nausea, vertigo, headache, thirst, vomiting and diarrhœa. Symptoms usually stop in 12 to 24 hours but may continue for several days. Urticaria during and immediately after the injection is comparatively rare. Herpes labialis and occassionally herpes zoster may occur. Adrenalin is also beneficial in these cases. Protein or colloidal shock produce fever, perspiration, chillness or even a rigor.

C. *Late reactions.* These occur from one day to several weeks after the injection. These are hæmorrhagic encephalitis, dermatitis, neuritis, Herxheimer's reaction, and jaundice. Hypersensitiveness to these reactions may be acquired. It is not infrequently seen that patients who have one or more courses of arsenical compounds, suddenly or gradually begin to develop immediate or early toxic reactions after small doses, *e.g.*, nausea, vomiting, flushing, occurring immediately after injections. The skin tests are negative so that the effect is not cumulative.

Herxheimer's reaction.

Herxheimer's reaction. These are of the nature of therepeutic shock and are characterised by exacerbation of the lesions and symptoms of disease following administration of arsenicals or penicillin. They occur more commonly with penicillin (in 60 to 80 per cent of case) than arsenicals. They vary in severity and occur within the first few days of administration of the drug. There may be only fever but in some case convulsions, transverse myelitis, hallucinations and mania may occur. Exacerbation of pains of tabes and acute gastro-intestinal reactions have been observed. Delayed reactions sometimes occur.

These reactions can be prevented by giving the drugs in small doses during the first 24 to 48 hours. This type of reaction has been attributed to liberation of ectic toxin from spirochætes killed by drugs. The luetic lesion especially of the trunk, show bright red erythema lasting a few days. Generally they cause little inconvenience, but analogous reactions in cerebral and hepatic gummata may be dangerous.

Cerebral symptoms.

Cerebral symptoms. Hæmorrhagic encephalitis occurs especially after large doses or when ordinary doses are given in quick succession. The symptoms start 2 to 5 days after injection with severe headache, vomiting, weakness, œdema of face, muscular twitching, dyspnœa, epileptiform convulsions, clonic spasms and suppression of urine. Loss of deep reflexes, a positive Babinski sign and continued convulsions are some of the signs of involvement of the central nervous system. Post mortem examination shows numerous small hæmorrhages, but no evidence of softening of the brain tissue; the capillaries are filled with hyaline thrombi. The lungs, spleen, and kidneys are all congested but free from thrombi. Arsenic is said to be responsible for the production of these symptoms. Hæmorrhagic encephalitis, however, commonly occurs in the second stage of syphilis. In a few cases it has occurred after a single injection of arsenicals, but in the majority of cases after the second injection. Examination of the brain may show the presence of arsenic or not. The ætiology of hæmorrhagic encephalitis is probably the same as that of the acute vasoparetic reaction following injection of arsphenamine.

Dermatitis and allied reaction.

Dermatitis and allied reaction. These are the most annoying of all reactions and consist of rashes on the skin and mucous membranes They are less common with organic compounds and their characters depend chiefly on the sensitiveness of the patient to the drug, the amount of arsenic given and the degree of skin reaction. The most common types are patches of urticaria, erythema and rarely purpura, sometimes there is only itching. The eruptions generally subside in a day or two. More persistent eruptions occur 6 to 10 days after administration, especially after intravenous injections. The severe type of reaction may turn into exfoliative dermatitis which is very serious, as death follows after its appearance in about 30 per cent of cases. Various stages may be passed so rapidly that an initial urticaria or discrete erythema may become a confluent erythema in 24 to 48 hours; later it may turn vesicular and subsequently pass on to exfoliative dermatitis. Arsenicals should therefore be discontinued if signs of skin irritation appear. Kolmer has classified these reactions into 4 groups.

1. Mild and early skin lesions consisting of simple erythematous rashes, urticaria, herpes, pruritus. These may be due partly to colloidal phenomena and partly to liberation of spirochætal endotoxins—Herxheimer's reaction.

2. Severe and late skin lesions consisting of dermatitis and purpura. The former may consist of scarlatiniform erythema with desquamation, erythema multiforme (papular and vesicular), lichenplanoid or with acute exfoliation which may be simple, rheumatic or hæmorrhagic.

3. Mucous membrane lesions consisting of stomatitis, exfoliative ileocolitis, conjunctivitis, vaginitis and broncho-pneumonia.

4. Chronic and recurrent skin lesions such as fixed arsenical rashes, pigmentation and argyria, melanosis, hyperkeratosis of the palms and soles of the feet may develop.

Forms of eruption.

Forms of eruption. 1. *Erythema.* This may vary from mild redness to deep redness with a purple tint. It blanches on pressure, appears first on the flexor surface of the limbs and trunk. It may be petechial or hæmorrhagic, but often it is either morbilliform or scarlatiniform, erysipelatoid or of a patchy character resembling a copaiba rash. The rash may be discrete and papular, though the urticarial form is more common; it may be accompanied by a pemphigoid eruption which is characterised by the formation of vesicles and blebs that leave considerable pigmentation behind.

2. *Exfoliative dermatitis.* This is preceded by an acute erythematous dermatitis and in a mild case it does not go beyond the erythematous stage. The infiltration is more noticeable on hands, feet and ears; vesicles or bullæ may form and rapidly rupture, thus producing areas of acute weeping dermatitis, marked redness of the skin, exfoliation, crusting

and scabbing from the drying exudate. The whole body may be uniformly affected or exfoliation may be confined to certain parts especially the ears, scalp, armpits, groins and fold of the skin. There is œdema of the eyelids, conjunctivitis and photophobia. Fever, headache, faucial congestion and emaciation are present. Secondary infection may take place. There may be loss of the hair of the scalp, eyebrows and eyelashes. The main complications are glandular enlargement, abscess formation in the groins, broncho-pneumonia, œdema of the lungs, exfoliative vaginitis. Albuminuria may be present. When recovery occurs convalescence is protracted, the skin becomes thin and atrophic and assumes a brown tint.

3. *Chronic skin lesions.* Fixed arsenical exanthemata develops by the production of urticarial patches in the same situation leading to an elevated smooth plaques. Raynaud's syndrome with gangrene has been recorded. Fibrosarcoma at the site of injection of arsphenamine has occurred.

The skin lesions are due to the direct effect of the arsenical compounds and not due to the syphilis itself, as they have occurred in non-syphilitic patients treated with arsenicals. The skins of patients with dermatitis contain more arsenic than those without. Pentavalent arsenic appears to have a special affinity for ectodermal structures, *e.g.,* the epidermis, sweat and sebaceous glands, hair, and relatively little affinity for blood vessels of the corium. Pentavalent arsenicals therefore produce mild dermatitis, keratosis, pigmentation, wrist drop and optic atrophy; while trivalent compounds cause severe dermatitis, encephalitis and purpura. The incidence of severe skin reactions is 1 in every 500 to 1,000 injections and sulpharsphenamine produces a somewhat higher incidence. Non-syphilitics may also develop it. The skin reactions are 2 to 3 times commoner in white races than in coloured races.

(ii) Treatment

Most cases of arsphenamine dermatitis present warning signs before the generalised dermatitis begins. In early cases, the treatment with arsphenamine should be guardedly continued by changing to a different type of arsenical, smaller doses, etc., before true allergic state develops. Physiologic solutions of sodium chloride, dextrose, gelatin, sodium thiosulphate and calcium have been added to the arsphenamine to render it less toxic. Clinical experience has proved that sodium thiosulphate is a very valuable detoxicating agent. Its mode of action is unknown but probably it transforms arsenic into a less toxic, more efficient and a more easily excretable product. On the other hand, some authorities believe that owing to the presence of the sulphydryl group, sodium thiosulphate changes the insoluble arsenic compounds developed in the skin into soluble forms. In order to avoid arsenical dermatitis it is advisable to combine sodium thiosulphate with arsphenamine. But once the dermatitis has set in, sodium thiosulphate fails to hasten the resolution; its value only lies in its use early in the disease. Fifteen grains (1.0 gm) of sodium thiosulphate by the mouth 3 or 4 times a day is said to produce good results, but in severe cases it should be given intravenously in 10 per cent solution, starting with 0.3 gm on the first day, then 0.45, 0.9, 1.2 and 1.8 gm on successive days. At the same time it is given by the mouth in 2 gm doses twice daily in 120 to 200 ccm of normal saline. Thiosulphate is said to act by releasing the arsenic deposited in the skin and along the nerve trunks until gradual return to normal is observed. The excretion of arsenic is accelerated, the parchment-like condition of the skin disappears and the pigmented areas become normal. Wassermann-fast syphilis is due in some cases to a saturation of the tissues by the heavy metals used in the treatment, or to arsenic-fast or mercury fast strains of spirochætes. In such cases sodium thiosulphate produces excellent result. A series of biweekly intravenous injections for 5 to 7 weeks, an average dose of 0.6 gm of the drug being given at each injection. The patient also receives 15 grains (1.0 gm) of this drug daily by the mouth at the same time. Sodium thiosulphate definitely alters the serum reaction in some cases of Wassermann-fast syphilis.

Thiosinamin has also been recommended intravenously in 3 grain doses in 10 ccm of sterile water but it may produce digestive disturbances, lassitude and fever. Intramuscular injections of intramine in doses of 2.5 ccm of a 1.0 per cent emulsion are beneficial; as many as 12 injections may be given.

Liver therapy.

Liver therapy. Based upon the hepatotoxic theory, some workers have used injections of liver extract in the treatment of arsenical dermatitis with good results. It is given in doses of 5 to 10 ccm intramuscularly, three times a week. The therapy stimulates the disturbed function of the liver, cures chronic arsphenamine intoxication and prevents further manifestation of arsphenamine sensitisation.

Other effects. An intense necrotic type of enteritis has followed treatment with arsenicals and mercury, leading to perforation and fatal peritonitis. Severe parenchymatous degeneration of the kidneys has been known to occur after arsphenamine. A true syphilitic nephritis is well known. Arsphenamine in rare cases may produce aplastic anæmia with degeneration of the bone marrow. There may be bleeding from the gums and mucous membranes in many cases and the red cell count may be reduced to 800,000 and hæmoglobin to 13 per cent, the leucocyte count is also reduced. Post-mortem examination shows complete aplasia of the bone marrow and broncho-pneumonia with a poor cellular reaction.

Syphilitic and arsenical jaundice.

Syphilitic and arsenical jaundice. The intensity of jaundice may vary from a transitory jaundice to acute yellow atrophy of the liver. It may be early or benign, late or severe, and lastly acute yellow atrophy may result. Early jaundice commences within a few days, sometimes within a few hours of the injection. It may come on after the first injection or after any subsequent injections. There may or may not be any constitutional disturbance, but that does not mean that the liver has not been damaged. Cases have been recorded with initial transient jaundice but some months later the patient has died of acute yellow atrophy of liver. Late jaundice is a very serious condition. It is accompanied by fever and usually occurs after many injections (one or two courses) and is believed to be more commonly caused by neo-salvarsan, though there are no data to support this belief. Jaundice is generally intense and unless there is acute yellow atrophy of the liver, the patient may recover. In the late type of jaundice symptoms are not so severe as in acute yellow atrophy. The jaundice, from the beginning is intense but non-febrile. Then suddenly symptoms of acute hepatic insufficiency, such as fever, rigors, vomiting, delirium and other nervous symptoms supervene and the patient dies of coma. Kolmer has classified jaundice into four groups according to its ætiology.

1. Due to arsenic, by production of fatty degeneration, hepatitis or obstructive jaundice by gastro-enteritis. That the arsenicals damage the liver can be shown by the lævulose and blood lipase test, even therapeutic doses produce structural alterations, and the phenol-tetrachlorphthalein test shows signs of insufficiency after a series of doses or courses. The interesting fact in connection with acute yellow atrophy is the long latent period usually intervening between the last injection and the onset of symptoms. With trinitrotoluene, jaundice may occur 2 to 10 months after exposure.

2. Due to syphilis alone. This may be mild or severe and the pathological changes are perihepatitis, diffuse hepatitis or gumma.

3. Due to arsenic and syphilis. This is considered to be a form of Herxheimer's reaction; syphilitic infection of the liver is intensified as a result of administration of arsenicals. Arsenicals may also stimulate a bacterial infection of the liver.

4. The immediate predisposition to jaundice is neither due to arsenic nor syphilis of liver, but in the majority of cases to summation of the changes produced by the disease and the arsenicals, and it is possible that the administration of mercury may help in intensifying the hepatic damage.

Prevention and treatment of jaundice.

Prevention and Treatment of jaundice. Rest in bed, mild laxatives, carbohydrate diet which increases the resistance of the liver, with no fats and little proteins are recommended. Milk may be given and the patient is advised to

drink plenty of water. When jaundice is clearly due to syphilis, give anti-syphilitic treatment with arsenicals, mercury or bismuth compounds; but if due to arsenic injections these should be immediately stopped. Intravenous injections of sodium thiosulphate, thiosinamine or contramine (diethyl-dithiocarbamate) have been recommended.

Meirowsky (1920) showed that when the dose of neo-salvarsan exceeded 0.6 gm the death rate went up considerably and hence he recommended 0.6 gm to be the maximum dose. The death rate was much less with neo-salvarsan than with salvarsan. Strong solutions of salvarsan and sulfarsenol are directly hæmolytic *in vitro* for washed corpuscles, but they are much less so in the presence of serum, and intravascular hæmolysis is not therefore very likely to occur.

Nervous reactions. Numbness of the fingers and the soles of the feet may occur. As these symptoms frequently forecast the onset of dermatitis the injections should be stopped. As a rule these are not accompanied by pain but this may occur and polyneuritis may be produced followed by paralysis. Neoarsphenamine is said to have produced more cases of paralysis than arsphenamine. Sometimes injections are followed several weeks or months later, by severe nervous manifestations, *e.g.*, epileptiform convulsions. They occur also with mercury and Ehrlich suggested that they are due to incomplete destruction of the parasites. The optic and auditory nerves are not affected by trivalent compounds but atoxyl and tryparsamide (pentavalent compounds) produce retrobulbar neuritis and transient dimness of vision or amblyopia. Arsenicals with an amino group or a substituted amino group in the para position to the arsenic, *e.g.*, atoxyl, produce optic atrophy in the rabbit. Organic arsenicals with the amino group in the ortho or meta position to the arsenic on the other hand, produce no optic atrophy. Herxheimer's reaction occurs in secondary syphilis especially if large doses have been given; small ascending doses of these compounds do not produce it. *Nervous reactions.*

Summary. The toxic symptoms produced by injections of organic arsenicals may be briefly classified as follows:—(1) Those occurring immediately, *e.g.*, flushing of the face, headache, lachrymation, œdema, dyspnœa, swelling of the lips, tongue and eyelids, nausea, vomiting and retching, unconsciousness and very rarely death (anaphylactoid or nitritoid crises). (2) Those appearing within 24 hours, *e.g.*, chilliness, rigors, headache, vertigo, diarrhœa, and rise of temperature. (3) Those observed after 24 hours, *e.g.*, epileptiform convulsions, dilation of the pupils, loss of reflexes, coma and rarely death. In addition there may be eruptions on the skin, exfoliative dermatitis (complicated with broncho-pneumonia and septicæmia), jaundice (early, late and acute yellow atrophy), epilepsy or hæmorrhagic encephalitis. Acute yellow atrophy often supervenes on late jaundice. Rare lesions are acute hæmorrhagic nephritis, ulcerative enteritis and aplastic pneumonia. (4) Complications now regarded as relapses of syphilis, are due to spirochætal toxins suddenly liberated. These include affections of the nervous system, deafness, cranial nerve palsies, etc. *Summary.*

Pathological changes produced by organic arsenicals. These changes were studied in animals. The type of changes produced is the same with all the trivalent and pentavalent compounds, some being more toxic than others. Tissue changes are chiefly due to the arsenic constituent and consist of, (1) congestion of capillaries and consequent serous exudation and minute hæmorrhages and (2) parenchymatous degeneration of organs and necrosis. If death occurs at once or within a few hours, there is widespread intense hyperæmia of the brain and meninges, lungs, liver, kidneys and other organs, with extensive thrombosis and plasma precipitates. Small capillary hæmorrhages are found in some organs, but degenerative lesions are not produced unless the animals survive a few days. In human subjects, where death occurs within 24 hours after accidental administration of acid solution

of salvarsan, advanced degenerative lesions are found in the liver, kidneys and other organs. The majority of deaths in human subjects have occurred after several doses have been given, death being due to chronic rather than acute poisoning. In Europe, salvarsan fatalities are mostly ascribed to hæmorrhagic encephalitis but in the United States they have been put down to degenerative changes in the liver and kidneys.

Cautions and contra-indications.

Cautions and contra-indications. The only real contra-indication is hypersensitiveness to arsenic. Individuals who develop acute reactions, dermatitis and jaundice are bad risks and in these cases other drugs should be tried. Sometimes the substitution of one compound for another may avert such reactions. In minor cases of dermatitis if a beginning is made with a small dose and it is gradually increased, severe reactions may be avoided. A sharp look-out must be kept for urticaria, fleeting erythema, itching of the palms, etc., as these give a warning that more severe reactions may follow. Cases of jaundice are also bad risks and very great care should be taken in giving arsenicals if previous injections have caused it.

Organic arsenicals should be given with caution in cases of emaciation, malnutrition, in diseases of the heart and blood vessels (syphilitic aortitis and myocarditis), in tuberculosis with hæmoptysis, in affections of the brain and meninges, in advanced cases of diabetes and nephritis, and in old and feeble persons of very advanced age. Arsenic should not be given where vitality is very low as a result of acute manifestations of syphilis. In syphilis of the optic nerve pentavalent arsenicals should never be given, while trivalent compounds should only be tried in small and ascending doses. According to some authorities tryparsamide may be given safely when the optic nerve is diseased provided the doses are small and carefully regulated.

Prophylaxis of toxic reactions.

Prophylaxis of toxic reactions. The measures taken have been divided into three groups.

1. *Those referable to the patient.* Allay fear and nervousness; if constipation is present it should be relieved; the injection should be given on an empty stomach, preferably in the morning; the urine should be examined previously in all cases, especially when mercury and bismuth are also being tried; the functional capacity of the liver may be tested in selected cases. The first dose should be small, ¼ to ½ of the usual dose; this tends to increase the tolerance of the individual. In long-standing cases, give mercury or bismuth first or very small doses of arsenicals to prevent Herxheimer's reactions. In women the injections should not be given during the menstrual period and special care should be exercised when pregnancy exists, as abortion may take place. When giving pentavalent compounds special attention should be paid to the eyes to guard against toxic amblyopia. The injections should be given deep into the muscles and not into the fatty tissue. In patients likely to develop vaso paretic symptoms 1/100 to 1/50 grain of atropine sulphate should be given half-an hour before the main injection. 2 to 8 minims of 1 in 1,000 adrenalin intramuscularly before the injection prevents acute vaso-paretic reactions. Adrenalin is the most useful drug to cope with 'nitritoid crisis.' Injection of calcium salts either prior to or in conjunction with salvarsan or neo-salvarsan injections is said to prevent the toxic effects of the drug. Calcium chloride dissolved in distilled water is usually given intravenously, but it may also be administered orally.

2. *Those referable to the preparation and administration of solutions.* Use freshly distilled water or saline free from organic matter. For dilute solutions (50 ccm or more) use 0.5 per cent saline in preference to water. Filter all solutions. After neutralisation allow arsphenamine solutions to stand for 10 to 20 minutes before injection. Injections should be given slowly. New rubber tubing should be avoided.

3. *Those referable to the drug.* Avoid products over two years old and all cracked ampules should be thrown away. If there is suspicion of a crack dip the ampoule in alcohol which will find its way in if there is a leak. Before injection carefully verify the drug which is going to be injected. Solutions should never be given unless they are brilliantly clear; cloudy solutions produce abdominal pains and immediate syncope. If necessary they should be filtered.

Keeping properties and media for solution. Salvarsan and the allied compounds should be kept sealed in ampules in vacuo. It is better to use fresh rather than old specimens. The Hygienic Laboratory of U.S.A. has placed a limit of six months on them from the date of preparation. Freshly distilled water should be used for making solutions. When concentrated solutions of neo-salvarsan are employed they should be dissolved in distilled water. A 5 per cent solution of glucose as a medium for dissolving these compounds reduces the toxicity by preventing oxidation in solution, at the same time it does not reduce their therapeutic activity. Neo-salvarsan and sulfarsenol are best dissolved in cold water. It has been clearly established that concentrated solutions (0.9 gm in 2 ccm) of neo-salvarsan and sulfarsenol do not do any harm when given intravenously. It has been pointed out that shaking the solution in air, even for a few seconds, increases the toxicity of all these compounds by oxidation but if contact with air is prevented by means of stoppered bottles, solutions may be safely kept for a few hours before injection; some say they may be safely kept for 24 to 48 hours. Excess of alkalinity or acidity of the salvarsan solution are both dangerous.

Keeping properties and media for solution.

7. Bismuth and its Derivatives

Bismuth compounds were used as cicatrising agents in the treatment of cutaneous lesions as early as the 17th century, and towards the end of the 18th century they were given internally for gastro-intestinal disturbances; bismuth oxide was used for dressing wounds and ulcers. Robert and Sauton (1916) tried bismuth preparations in the treatment of spirochætosis of fowls and found them to be effective. Sazerac and Levaditi (1921) found that in experimental syphilis of rabbits bismuth compounds had a well-marked curative action. They tried intramuscular injections of tartro-bismuthate of sodium and potassium in syphilis in man and found that though the injections were painful, the drug produced rapid cicatrisation of the lesions of this disease. Further trials showed that primary chancres healed rapidly, secondary lesions were inhibited and early secondary and tertiary lesions disappeared after 10 to 12 intramuscular injections of bismuth.

These findings led gradually to the introduction of bismuth in the treatment of syphilis. As an adjunct to the arsenobenzene derivatives, bismuth is now recognised as a drug of considerable value and has replaced mercury in the chemotherapy of syphilis.

(1) Pharmacological Action

(1) *Pharmacological action.* Like arsenic and antimony, bismuth in very high dilutions exerts a markedly toxic action on certain pathogenic protozoa; 1 in 200,000 will kill some of the organisms *in vitro* and it is possible that in the body this action may take place even in lower concentrations. Like atoxyl, these compounds only become active when incubated with liver extract and to a lesser extent with extracts of other tissues.

External. Soluble bismuth salts are not used externally. The insoluble basic bismuth salts are largely used as dusting powders on inflamed or irritated surfaces. Their action is mainly mechanical as protective, drying and dusting powders. Applied to wounds, they dry secretions, form a protective covering and exert an astringent and antiseptic action. Sometimes absorption from the wounds and raw surfaces may be very rapid giving rise to symptoms of poisoning.

External.

Internal. *Internal.* Bismuth compounds have the reputation of being antacids. This effect is partly due to diminution of gastric secretion and partly to neutralisation of the gastric acidity. In the intestines both the subnitrate and the subcarbonate neutralise the sulphides of alkali metals and hydrogen sulphide, which produce irritation and increased intestinal peristalsis; these bismuth salts thus act as gastro-intestinal sedatives in inflammatory conditions of the gut. Further they are believed to coat the surface of the inflamed mucous membrane and allay irritation by preventing the irritant gases and fluids from coming in contact with the mucosa. They also exert a certain amount of antiseptic and astringent effect and are therefore prescribed in diarrhœa. Subcarbonate of bismuth is preferred to subnitrate as the latter is converted into nitrous acid by reduction in the intestines. In the gut bismuth salts are converted into sulphide, which is black in colour; it may form crystals looking like hæmatin and may be mistaken for it. Bismuth has been combined with other substances either in mixture or in synthetic compounds to produce insoluble compounds with enhanced protective and antiseptic actions, *e.g.,* bismuth subgallate or bismuth salicylate but this is doubtful.

Circulation and respiration. *Circulation and respiration.* After intravenous injections, even in small doses, there is a fall of blood pressure, partly due to depression of the vasomotor centre, but chiefly to the direct effect on the heart which becomes slow and irregular. The respiration is accelerated at first, but later depressed. The blood shows a mild and temporary leucocytosis after injections of bismuth salts.

Kidneys. *Kidneys.* All bismuth compounds tend to increase the output of urine and thus promote their own excretion. The administration of water and sodium chloride, etc., hastens the excretion of bismuth. A high chloride dietary would, therefore, favour the efficiency of bismuth medication by helping the mobilisation of the metal from the tissues. The diuretic action of bismuth may be made use of in the treatment of œdema and anasarca. Its action has been found to be well sustained and useful in the removal of large quantities of fluid.

Central nervous system. *Central nervous system.* In frogs the symptoms are those of stimulation of the spinal cord and medulla oblongata, followed by depression and paralysis. In mammals, intravenous injections of large doses of bismuth act chiefly on the central nervous system producing violent clonic and tonic convulsions, followed by short intervals during which the movements are weak and incoordinated.

Absorption of bismuth. *Absorption of bismuth.* Only very minute quantities of bismuth are absorbed from the alimentary canal. This is shown by the fact that poisoning never results even when large doses of bismuth compounds are given by the mouth if the mucous membrane is intact. If there is solution of continuity and ulcers are present, the drug may be rapidly absorbed.

Absorption is so slow and uncertain from the gastro-intestinal tract that this route of administration is not employed for the treatment of such diseases as syphilis where a systemic action is desired. Hypodermic injections are too irritant and are not advisable. Intravenous injections of most of the bismuth compounds are not considered safe. This can be judged from the fact that while intramuscularly a rabbit can survive 200 mgm of bismuth salts per kilo body weight, 5 mgm is sufficient to kill it if given intravenously. Intramuscular injection is therefore the only mode of administration and is universally adopted.

Fate of bismuth in the body. *Fate of bismuth in the body.* After absorption, bismuth like antimony, is widely distributed in the tissues of the body but in unequal amounts. After intramuscular injections bismuth can be detected in the blood in fairly high concentration in the plasma, though the corpuscles also contain it in negligible amounts. The concentration is highest in the kidneys indicating that the kidneys can store bismuth. The long bones, liver, lungs, spleen, skeletal muscles and the brain also contain traces of the drug. The placenta is permeable to bismuth and it can be found in the fœtus. There is improvement in the condition of many neuro-syphilitics following bismuth injections and minute quantities probably reach the central nervous system.

Bismuth appears to have the same distribution as other heavy metals. Owing to its widespread distribution syphilitic lesions of different parts of the body improve after bismuth therapy.

Excretion. *Excretion.* Although some bismuth is stored in the liver and other organs, the greater part is eliminated by the kidneys, liver and intestines, only traces occur in sweat, milk and tears. The main channel of excretion after injection appears to be the kidney, only one-twelfth to one-eighth of the bismuth appears in the fæces. Bismuth appears in the urine within 18 to 24 hours after intramuscular injection of 'trepol' and excretion is continued for 20 to 25 days after a course of treatment in which 2.0 to 2.5 gm have been injected. After intravenous injections bismuth can be detected in the urine in four hours. Sometimes the urine when voided is discoloured or becomes so on standing. In the latter case this is due to the formation of bismuth sulphide by the action of bacteria. The diuretic action of bismuth salts has already been referred to.

In human poisoning, bismuth is found mainly in the stomach and kidneys, and a little in the liver. Autopsy shows the cæcum, adjoining colon and appendix stained black with bismuth sulphide, which is deposited in the mucous membrane in the capillary vessels and lymph spaces. The small intestines are clear.

(2) Therapeutic Uses of Bismuth

Bismuth subnitrate and carbonate are valuable drugs in the treatment of inflammatory conditions of the intestines, in diarrhœas and in gastro-duodenal ulcers. They should be given in large doses, 30 gr (20 gm), as a powder or in suspension, by itself or in association with magnesium oxide or sodium bicarbonate on an empty stomach. Bismuth compounds act well in gastralgia and hyperchlorhydria and combat flatulence. In combination with alkalies they are also used as accessories to the emetine treatment of amœbic dysentery. They also ameliorate symptoms of this disease by their inhibiting action on the peristaltic movements of the intestine by neutralising the alkaline sulphides and sulphuretted hydrogen present there. They are used in chronic ulcers of the skin and in the treatment of deep-seated sinuses. The nitrate or carbonate of bismuth, mixed with vaseline in 30 per cent strength is injected into tuberculous and other sinuses with the object of producing healing effects by cicatrization. They are however liable to cause poisoning if the sinuses are very extensive.

Syphilis. The bismuth treatment of syphilis is one of the noteworthy advances of therapeutics. The spirochæticidal properties of the organic and inorganic compounds of bismuth have been thoroughly tested and they are being largely used at present in the treatment of syphilis. *Syphilis.*

Bismuth compounds are found to possess spirochæticidal activity of a very high order. This will be appreciated from the fact that a concentration of 0.2 mgm in one litre of blood is sufficient to produce an antisyphilitic effect. A single therapeutic dose is five times smaller than the single maximum tolerated or toxic dose, *i.e.*, the chemotherapeutic index is 5, which is very much higher than that of mercury compounds, but not so high as that of the organic arsenicals.

All forms of clinical manifestations of syphilis respond to bismuth treatment. The effective curative dose is 4 to 5 mgm per kilo per course of which two, three or more may be given. In primary and secondary syphilis a few centigrammes of tartro-bismuthates produce rapid disappearance of spirochætes from the lesion, and cicatrization. Spirochætes disappear from the chancre in 24 to 56 hours, the chancre is healed in 4 to 10 days, and enlarged lymph glands in the neighbourhood of the chancre disappear. Rapid disappearance of mucous patches occurs after administration of bismuth compounds; a previously positive Wasserman reaction may become negative under treatment but not so rapidly as with the arsenicals. With colloidal bismuth mucous papules become cicatrized and treponema disappear after the first or second injection. Headache, fatigue and pains in the bones frequently cease after a few injections. In the tertiary stage of the disease bismuth is also useful, gummata disappear and also chronic encrusted ulcers of long standing and ulcers of the palate heal up rapidly. Cases which are resistant to mercury and arsenic, sometimes do better with bismuth. Bismuth appears to be less effective and slower in action than salvarsan but more effective than mercury. After treatment with arsphenamine no living spirochætes can be detected after 24 hours, whereas after treatment with bismuth, living spirochætes do not disappear for 3 to 4 days in the mucous lesions. Bismuth is not so depressing as mercury, old and debilitated patients tolerate it better than arsenic, and it can also be given for a much longer period than either mercury or arsenic. Bismuth is said to be specially effective in syphilitic lesions of the eye

and in congenital syphilis. That reinfection after bismuth therapy has occurred shows that the sterilization produced by it is definite. Simultaneous treatment with arsphenamine is not necessary for clearing and healing of acute lesions, or for sterilisation of lymph glands, as bismuth is effective by itself. Combined treatment however is desirable to cut short the length of treatment and to diminish the discomfort and inconvenience of the patients. The combination of bismuth with arsenicals is now preferred to the combination of mercury and arsenicals because the former is more effective.

ffect on rological actions.

Effect on serological reactions. The development of a positive reaction in case of primary sero-negative syphilis treated with arsenicals is rare; but with bismuth treatment a weakly-positive reaction may develop. After one month's treatment of primary and secondary sero-positive cases more sero-negative cases are obtained with arsenicals than with bismuth preparations. Cases of positive Wassermann reaction, which are uninfluenced by arsenic, are unaffected by bismuth also. In congenital syphilis there is said to be more chance of the reaction becoming negative with bismuth than with arsenicals. The action of bismuth is considered by some to be inhibitory rather than curative.

eurosyphilis.

Neurosyphilis. Syphilis of the nervous system is not frequently benefited by arsenic preparations; in these cases bismuth preparations are useful. Their efficiency in the neuromanifestations appears to be superior to that of the arsphenamines and equally as good as that of mercury. Tabetics are generally benefited by bismuth compounds, symptoms of gastric crisis and urinary incontinence may be ameliorated and ataxia may show some improvement. Insoluble preparations in the form of intramuscular injections are recommended in these cases, especially when mercury is not well borne. Bismuth is proving more useful than mercury in the treatment of meningeal neurosyphilis with headache. Mercury was the chief drug used in the treatment of neurosyphilis and even now it is advised by the neurologists in combination with arsenical treatment.

Bismuth has established for itself a permanent place in the treatment of syphilis by reason of its low toxicity for the body associated with marked spirochæticidal properties; it is specially indicated in the treatment of acute syphilis when arsphenamine and its substitutes cannot be given, *e.g.*, in nephritis, jaundice, etc.; it is of value in the treatment of chronic syphilis and especially that of the central nervous system. It should be used in the treatment of syphilis in all its stages along with organic arsenicals as a form of combination therapy, which is the keynote of success in the treatment of either acute or chronic syphilis. It is specially indicated in cases resistant or intolerant to arsenic.

Prophylactic effects of bismuth in syphilis. The value of mercury applied locally after exposure to infection has been known for a long time. Experiments on apes and man have shown that inunction with calomel cream prevents development of the infection. In rabbits calomel in lanoline, vaseline, benzoated lard or wax base, proved efficacious up to eight hours after inoculation.

Bismuth, like mercury and arsenic, is also claimed to have a prophylactic value against syphilitic infections. It has been seen that in experimental syphilis bismuth acts definitely as a prophylactic and that bismuth medication protects rabbits from infection for a month or more.

Yaws or Framboesia. As a result of its successful use in syphilis, bismuth has naturally been employed in the treatment of yaws. Extensive trials in British East Africa have shown that bismuth compounds are nearly as efficacious as neo-salvarsan. Two or three injections of tartro-bismuthates in doses of 0.2 gm in freshly prepared solutions at intervals of a week produce healing of the lesions,

but a larger number will be required to eradicate the parasites. Besides bismuthyl tartrates, bismuth subgallate (dermatol) has been used in 3 ccm doses of a 10 per cent emulsion in oil given intramuscularly twice a week. Very good results were obtained with 10 injections, but this produced toxic effects. On a large scale patients have been treated with a single massive dose of dermatol; only 15 per cent relapsed. The author has tried *bisnene* in doses of 0.1 to 0.15 gm intravenously in a few cases with good results. The results are as good as those with neo-salvarsan and other bismuth preparations; it is much cheaper, more stable and costs less than a farthing a dose.

Lupus erythematosus. This a chronic non-tuberculous affection of the skin characterised by disc-like patches on the surface with reddish edges and depression at the centre. The patch is covered with scabs which fall off gradually and leave a dull white cicatrix at the bottom. The etiology of the disease is unknown. Bismuth salts have been used in this condition with good results. Bismuth metal, bismuth hydroxide, bismuth oxychloride, or sodium bismuth thioglycollate suspended in oil is given intramuscularly once a week. The average dose ranges from 0.3 to 0.4gm. Local treatment with mercury improves the condition in many cases.

(3) Modes of Administration

By the mouth. As already stated, only very minute quantities of bismuth are absorbed from the alimentary canal. The absorption is so slow and uncertain that this route of administration is not employed for the treatment of such diseases as syphilis.

By the mouth.

A preparation named sobisminol has been introduced which can be given by intra-muscular injection or by mouth. It is given in capsules each of which contains 0.2 gm of sodium bismuthate. Orally the drug is well tolerated and rapidly produces involution of the lesions in early and benign late cases. It causes disappearance of spirochætes from surface lesions of early cases. This is a valuable addition to syphilitic therapy if further trials prove its efficacy.

Subcutaneous and intramuscular injections. Subcutaneous injections of the soluble salts usually produce severe local reactions and, as they have no advantage over the intramuscular route, this route cannot be recommended.

Subcutaneous and intramuscular injections.

Tartarobismuthates of sodium and potassium and metallic suspensions of bismuth are given intramuscularly with a thick needle in the superior gluteal region. Injections are given alternately on either side, not oftener than once a week as there is danger of cumulative poisoning.

Local reactions due to muscle irritation and necrosis may follow intramuscular injections. Pain and inflammation vary according to the nature of the compound used and the vehicle employed. The common type is a hard and painful swelling with considerable infiltration which may sometimes suppurate. The addition of 10 minims of a 2 per cent butyn solution or phenol (1/5 gr) to trepol reduces the pain effectively though an area of tenderness may be left. Histological examinations five days after injection show varying degrees of necrosis of muscle cells and leucocytic infiltration. Watery solutions produce a more intense reaction than the oily solutions. Nodules in the tissues left after intramuscular injections of bismuth compounds are due to purely inflammatory reactions and are less likely to be produced when solutions are made in vegetable oils such as olive or almond oil.

Intravenous injection. The intravenous injections of soluble bismuth salts are so toxic that they cannot be recommended. The toxicity by this route is at least ten times greater than the intramuscular route.

As in the case of other heavy metals, agglutination and hæmolysis may occur after intravenous injections, with the formation of emboli. Even with such compounds as tartrobismuthates, colloidal preparations or bismuth hydroxide, reactions may be produced immediately after injections. Cases of sudden death after intravenous injection of bismuth compounds with symptoms of colloidal shock have been reported. Mercury is more toxic by the intravenous route than bismuth, the maximum tolerated dose of perchloride of mercury is 0.006 gm per kilo. while that of the soluble tartrobismuthates is 0.02 to 0.03 gm. Some authorities have shown that the danger of agglutination of erythrocytes is small, but bismuth may produce precipitation of serum-proteins in the same way as do the arsenicals or antimonials.

(4) Toxic Effects of Bismuth

The toxicity of bismuth compounds has been worked out on animals. The earliest symptoms following intravenous injections of bismuth salts in experimental animals are loss of appetite, nausea, vomiting and diarrhœa; salivation and stomatitis with ulceration of the gums, tongue and buccal mucous membrane appear shortly afterwards. Weakness, slowness and inco-ordination of movements follow and tetanic convulsions may occur at intervals. The urine contains albumin and casts. The weakness gradually deepens into complete paralysis and the animals generally die. Post-mortem examination shows congestion and sometimes necrosis of the kidneys. Congestion and a black discolouration of the cæcum and upper part of the large intestine are also prominent features. The mucous membrane may show areas of patchy hæmorrhage, ulceration or necrosis. The histological changes produced in the kidneys and liver of rabbits, suffering from bismuth poisoning, following the administration of tartro-bismuthates, have been studied. Such experiments show, (1) that intramuscular injections are less toxic than intravenous injections and (2) that bismuth is most toxic for the kidneys, next for the liver and relatively non-toxic for the brain, heart, lungs, suprarenals and spleen.

Changes in human beings appear to be of the same nature, and symptoms are similar to those produced in animals. The toxicity of bismuth is comparatively low, undesirable effects are comparatively few and generally not serious. In view of the extended use of bismuth in syphilis, these will be given in detail.

Symptoms. After intramuscular injection of tartro-bismuthates, the earliest symptoms observed are pain in the muscles and joints accompanied by loss of appetite and feelings of malaise and lassitude. These, as a rule, are not severe, but in exceptional cases intense pain in the larger bones, joints and groups of muscles, accompanied by rigors, fever, a feeling of compression in the chest and dyspnœa may occur a few hours (12 to 24 hours) after the injection. Rheumatoid pains of the ribs, spine and legs are characteristic; skin eruptions of the nature of erythrodermia or scarlatiniform erythema with purpura may occur. Jaundice is as a rule not met with and bismuth may be given to patients in whom arsenicals have produced jaundice. Local irritation at the site of intramuscular injection is not uncommon; a 'lead-line' may occur at the junction of the teeth and gums due to precipitation of bismuth sulphides; a blue line in the gums, odorous breath and bad taste in the mouth often precede it. Blue spots may appear under the tongue and in the mucous membrane of the cheeks; gingivitis is caused by the deposition of bismuth in the capillaries, producing partial blockage of vessels,

congestion and loss of nutrition and formation of ulcers. Ptyalism is unusual and when present is not so severe as in the case of mercury. Bismuth is less likely to produce renal irritation than mercury, and albuminuria is therefore very uncommon even after 12 to 15 intramuscular injections of 0.1 to 0.2 gm each of tartro-bismuthates. It is however advisable to examine the urine every week during the course of injections. Herxheimer's reaction, *i.e.*, pain and swelling of syphilitic lesions in the lymph glands, skin, etc., are sometimes seen after 2 or 3 intramuscular injections; vague pain in the heart and other parts of the body, which are probably due to provocative effects, sometimes occur. These effects however are not so common as with the arsenicals.

Toxic symptoms produced in human beings by the oral administration of bismuth subnitrate may be due, not to bismuth but to the nitrate portion which is reduced in the gut to nitrite and produces symptoms of nitrite poisoning. After a large dose of this compound for radiography, methæmoglobinæmia, intense cyanosis, diarrhœa, and collapse may occur; death may even occur from paralysis of respiration. All these can be prevented by using bismuth subcarbonate instead. Considerable absorption of bismuth may however take place from wounds and raw surfaces and symptoms of acute or chronic poisoning may follow. Capillary thrombosis from precipitation of bismuth sulphide in the intestinal vessels has been known to occur.

Intramuscular injections and injection of bismuth compounds into long sinuses sometimes give rise to toxic phenomena, from excessive absorption of the metal. This condition of the patient is an important contributing factor. If the mouth and the gums are in bad condition there may be gingivitis, stomatitis, and halitosis (offensive breath); if the patient is a victim of gastro-intestinal disorders, he is more likely to suffer from diarrhœa and colic; focal infection of the teeth, tonsils and other tissues may predispose to dermatitis. Some individuals are naturally hypersensitive to bismuth. Four factors are concerned in the production of toxic symptoms, (1) the drug, (2) the route of administration, (3) predisposing pathological state of individual patient and (4) hypersensitiveness to bismuth.

The circulatory depression, that is noticed in animals receiving intravenous bismuth, does not occur after the clinical use of bismuth as an anti-syphilitic. Sometimes acute reactions due to colloidal shock may occur. The symptoms complained of are dizziness, pallor, rapid and weak pulse, dyspnœa, convulsions and collapse. Siderosis, halitosis, ptyalism, and gingivitis are commonly observed; various skin eruptions (purpuric, scarlatiniform, erythematous, urticarial, lichenoid, exfoliative dermatitis), pruritis, renal irritation and provocative Herxheimer's reactions are sometimes seen. Stomatitis and gingivitis are the most troublesome symptoms.

The mechanism of production of siderosis, stomatitis, and allied lesions has been explained, but the muscle and bone pains are more difficult to interpret. These are probably due to a neuritis similar to that produced by arsenic and mercury.

Treatment of toxic reactions. In poisoning from large doses of bismuth compounds by the mouth, the stomach is washed out thoroughly and repeatedly and saline purgatives are administered. If bismuth paste has been given in a sinus it should be removed.

Treatment of toxic reactions.

In case of acute poisoning due to a large accidental dose, the most dangerous lesion is tubular nephritis. If this is produced intravenous injections of sodium thiosulphate are indicated. As a rule muscular pains, malaise and anorexia disappear after a few injections, but if they persist and there is anæmia, loss of weight or ulceration of the gums, the treatment should be stopped. For gingivitis and stomatitis a mouth wash containing alum and potassium chlorate is the best. Ulceration of the gut is relieved by proper dietary and demulcents.

Cautions and contraindications.

Cautions and contraindications. Before beginning a course of bismuth a thorough investgation must be made into the condition of the teeth, the liver function and the general health of the patient. If there is any such defect, refrain from giving the treatment till these have been attended to. Cleansing of the teeth and gums during the course is important; dietetic errors should be corrected to reduce the possibility of gastro-intestinal disturbances, such as diarrhœa and colic; the appearance of large quantities of albumin and casts in the urine also calls for suspension of treatment; if albumin had been present prior to treatment, soluble salts are better. The urine should always be examined before starting bismuth injections and during the course. In exceptional cases renal function tests may be necessary but liver function tests are not required. Sometimes a general asthenia with pallor and loss of weight occurs after bismuth injections, but this soon passes off.

Mode of action of bismuth compounds. The mechanism by which bismuth compounds produce their spirochæticidal action *in vitro* and *in vivo* is not clear. Tartro-bismuthates of sodium and potassium are capable of destroying the motility of *S. pallida* in very high dilutions, and bring about their complete destruction when given by inoculations in rabbits. In both these respects mercuric chloride and mercurochrome are more active than bismuth compounds. Levaditi believes that bismuth enters into a colloidal state and exerts a direct spirochæticidal effect in this condition. The mechanism is probably similar to that of mercury and is largely dependent on the ease with which the bismuth ions are dissociated. There is no evidence to show that new compounds are formed by processes of oxidation or reduction, or that bismuth increases the production of spirochæticidal antibodies. It is possible that the actual destruction of the parasites is due to the union of the metal with the proteins of the parasites. According to Eagle (1938-9) there is no evidence that tissue derivatives in any way contributed to the *in vitro* antispirochaetal activity of bismuth. According to him bismuth acts niether as a catalyst, nor as inhibitor nor a precursor substance, but acts directly on the parasite as is the case with arsenoxide.

Amoebiasis.

Bismuth is also used as a non-specific adjuvant in intestinal amœbiasis and its main action is as a demulcent protective astringent. It reduces intestinal putrefaction and hastens degenerative changes in the entamœba by altering their environment. For this purpose bismuth subcarbonate is given in doses of one heaped teaspoonful (12 gm or 3 drams), stirred up in a glass of water or milk, every four hours till improvement results. It is said to coat the ulcers and affords them mechanical protection. Other compounds used for this purpose aie bismuth subnitrate B.P. and bismuth subgallate.

(5) Bismuth Compounds

The compounds of bismuth proposed for obtaining systemic effects of bismuth in the treatment of syphilis have been divided into following groups:—

(1) *Sobisminol Mass* is an interaction product of sodium bismuthate, triisopropanolamine and propylene glycol. It is a complex compound which contains 19.25 to 20.25 per cent Bi; 0.75 gm of sobisminol mass equals 150 mgm of Bi. It is used for oral administration in cases in which intramuscular injections cannot be given or the patient is away from the physician; also in congenital and latent syphilis. It is, however, a dangerous drug for patients to use without medical supervision. Appearance of toxic symptoms especially gastro-intestinal, should be particularly watched. Absorption of drug is rapid and sufficient to maintain effective concentration of Bi in the body and its curve of urinary excretion closely resembles that from intramuscular injection of water and oil soluble compounds. The compounds can also be given by intramuscular injection. The toxicity of the compound compares favourably with other water-soluble compounds and there appears to be no tendency to cumulative toxic effects.

Dosage:—It is given in capsules each representing 150 mgm of metallic bismuth; two or three capsules are given three times a day with plenty of water in adults. For children the dose is half or less.

(2) *Water soluble compounds.*

These are used in aqueous solution; some of these are also used in oil.

(a) *Bismosol* is potassium sodium bismuthotartrate in 10 per cent sterile glucose solution (0.1 gm in 1 ccm); it contains a preservative; Bi content 35 mgm of solution. Dosage 1 ccm three times a week till twenty doses have been given. Second course after one month's interval.

(b) *Bismuth sodium tartrate* (contains 72.7 to 73.9 per cent Bi); 0.3 gm of drugs in 0.04 gm of benzyl alcohol in 2 ccm of 25 per cent sucrose solution (contains 11 mgm Bi); dose 2 ccm (0.03 gm) twice or thrice weekly.

(c) *Thio-Bismol* is sodium bismuth thioglycollate, 0.2 gm to be dissolved in 1 ccm of distilled water (contains 76 mgm Bi.) Dose 1 ccm 2 or 3 times weekly.

(d) *Sobisminol solution.* A complex organic compound (each ccm containing 19.5 to 20.5 mgm Bi). Dose 2 ccm intramuscularly twice weekly in adults, for 20 to 25 injections.

(e) *Bismocymol* (37 to 40 per cent Bi) is basic Bi salt of camphocarboxylic acid in olive oil, each ccm containing 0.125 gm of drug. Mixture contain 50 mgm Bi. dose 2 ccm (0.25 gm) twice weekly for eight to ten injections.

(f) *Potassium bismuth tartrate.* A 10 per cent suspension in peanut oil with 0.4 per cent butyn; each ccm contains 0.1 gm of drug; mixture contains 66 mgm Bi. Dose 2 ccm (0.2 gm) weekly.

(g) *Bismuth oxychloride.* A 10 per cent solution with dextose and chlorocresol in water, each ccm containing 0.1 gm of drug. Dose 1 to 2 ccm twice weekly.

(h) *Sodium Iodobismuthite.* Consisting chiefly of hydrated Na_2BiI_5, it contains about 21 per cent of Bi. Dose 1 to 2 ccm twice weekly.

(3) *Water insoluble compounds used in oil suspensions.*

(a) *Bismuth subsalicylate.* A basic salt containing 62 and not more than 66 per cent of Bi_2O_2. A 10 per cent suspension in peanut oil, each ccm containing 0.1 gm of drug; mixture contains 57 mgm Bi. Dose 2 ccm (0.2 gm) weekly.

(b) *Oleo-Bismuth* is bismuth compound (camphorate, camphocarbonate butyl thiolaurate etc.,) suspended in olive oil, each ccm containing approximately 0.25 gm of drug; mixture contains approximately 50 mgm of Bi. Dose 2 ccm once or twice weekly.

(c) *Mesurol* is basic bismuth methoxy hydroxy benzoate 20 per cent suspension in sesame oil each ccm containing 0.2 gm of drug; mixture contains 110 mgm of Bi. Dose 1 ccm weekly.

(d) *Quinobine* is quinine-bismuth iodide rendered soluble in olive oil by lecithin, each ccm containing 0.158 gm of drug; mixture contained 30 mgm Bi. Dose 1 to 2 ccm (0.16 to 0.32 gm) twice weekly.

(e) *Tartro-quinioline.* Suspension of quinine-bismuth iodide 0.072 gm and sodium potassium bismuthyl tartrate, 0.033 gm in 1 ccm of olive oil. It contains 25 mgm of Bi. Dose 1 to 2 ccm weekly. Besides these *Iodobismitol with Saligenin* (dose 2 ccm twice weekly). *Iodobismitol with benzocaine* (dose 2 ccm repeated every three days) are in use.

Bismarsen is a combination of sulpharsphenamine and Bi; it contains 12 to 15 per cent of arsenic (trivalent) and 23 to 25 per cent of Bi. It is sold in ampoules and sterile distilled water is added directly into the ampoule; 1 ccm for 0.1 gm. The solution is given intramuscularly in average doses of 0.2 gm every three or four days. It is, however, not so effective as neo-arsphenamine and easily gives rise to toxic symptoms.

(6) Summary

Bismuth has both a spirochaeticidal and spirochaetostatic effect. Best results have been obtained with intramuscular injections; those compounds which can maintain the therapeutic level of Bi in the blood as reflected by urinary excretion, probably being the best. Intravenous injections are contraindicated as therapeutic doses closely approach the toxic doses. Intramuscular injections are given by inserting the needle in the inner angle of the upper and outer third of the buttocks, deep down into the muscular tissue. With the Syringe tip inserted into the needle the plunger is aspirated back a little to be sure that the needle is not in a vein or an artery; this will prevent venous and arterial emboli.

The efficacy of bismuth compounds lies midway between the arsenicals and mercurials. Combined Penicillin and bismuth therapy gives the best chance for a cure. The penetration of bismuth into the nervous system has not yet been definitely demonstrated by its presence in the cerebro-spinal fluid. Watery

solutions as a rule, if repeated often enough (2 or 3 times a week) give rapid absorption of metal and a sustained high concentration in the blood. Oil suspensions have a slower rate of absorption and concentration in blood stream, thus requiring only one injection a week. Certain oil suspensions, however, are more quickly absorbed than others, *e.g.*, the absorption of bismuth subsalicylate is slowest and its excretion goes on for a long time. It is, however, doubtful whether excretion indicates a therapeutic level.

8. Mercury and its Derivatives

Mercury was the first drug to be used as a specific disinfectant for the body tissues, for it was employed in Europe in the treatment of syphilis as early as 1,500 A.D., and until the discovery of arsphenamine in 1,905 it remained the only effective remedy against this disease. As a therapeutic agent in syphilis it has now been entirely replaced by bismuth. Mercurials have however a powerful destructive action on some of the pathogenic protozoa and bacteria and a number of new mercury compounds have been introduced in medicine during recent years.

(1) Pharmacological Action

External action. Mercury has a powerful toxic action on all protoplasm and mercurial compounds, and for this reason, are active germicides. This germicidal effect depends chiefly on the concentration of mercuric ions in their solution, and on the precipitation of proteins. Absence of protein precipitation is important because it diminishes local irritation, favours the penetration of the mercury and obviates the absorption of the mercury ions by the precipitates.

A large number of mercury compounds are used externally for their antiseptic and germicidal properties.

Heart. The inorganic salts and so also the organic compounds, such as mercurochrome, rapidly produce cardiac irregularities and fibrillation. The response of the isolated heart to the action of mercury varies in different animals. The frog's heart is very resistant to the action of mercury and a concentration as high as 1 in 50,000 is necessary to show any marked effect. The turtle's heart responds in as low a concentration as 1 in 1,000,000. In all cases mercury produces depression, irregularity and delirium cordis. Recent work has however shown that the mercury ion in a high state of dilution has a definite stimulant action on animal tissues. A 1 in 1,000,000 concentration of mercuric chloride added to the perfusate distinctly stimulated the isolated mammalian heart and increased its force of contractions.

Gastro-intestinal tract.

Gastro-intestinal tract. Mercury, by whichever route it is administered, exercises a selective action on the gastro-intestinal tract and increases the normal peristaltic movements. When soluble salts are administered orally, the effects on the mucous membrane are very severe. Hyperæmia, redness and swelling are immediately produced which commonly end in ulceration of the mucous membrane. The symptoms associated with such lesions are generally diarrhœa with rice water stools, intense pain, tenesmus and passage of blood and mucus with the stool. The insoluble salts like calomel dissolve so slowly and to so limited a degree that the irritative phenomena observed with the soluble salts are not manifested but catharsis is produced. Calomel is widely employed for this purpose and it produces a soft stool without pain or griping.

Mercury has very little action on the ferments of digestion. The antiseptic action on the intestine, especially of calomel, is due to retardation of putrefaction and decomposition of foodstuffs by increased peristalsis that hurries the contents down the intestinal canal.

Kidney.

Kidney. The kidneys are stimulated with small doses and diuresis is produced. The mechanism of mercurial diuresis has been explained on the basis of a mild renal irritation, which favours filtration through the glomeruli and probably hinders reabsorption in the tubules. Large doses of mercury produce characteristic changes in the kidney in a few hours with consequent anuria, cloudy swelling and necrosis of the epithelium of the tubules. The congestion of the glomeruli and the necrosis of the tubules form the prominent features in post-mortem appearances.

Central nervous system. The action on the central nervous system is not marked in therapeutic doses. Tremors and giddiness may be temporarily produced. Workers in mercury mines, in mirror works, barometer and thermometer factories are specially liable to develop poisoning symptoms from absorption of mercury. These symptoms consist in great muscular weakness which may develop into hallucination and delirium. Another feature of the nervous affections is a peculiar tremor which involves the hands and arms, and ultimately the legs. Shooting pains along the course of the nerves and joint pains have also been observed. The higher centres of the brain are rarely affected by mercury. In chronic poisoning, signs of hallucination and delusion may point to an affection of the cerebrum but they are extremely rare.

Metabolism. The effects of mercury on metabolism are not fully understood, but in general resemble those of arsenic and phosphorus. Small doses are said to improve the nutrition and lead to acceleration of metabolism of proteins. Prolonged administration may give rise to cachexia.

Fate in the body. Metallic mercury and mercury compounds are readily absorbed from all surfaces including the intact skin. Complex chemical changes are said to occur in the course of absorption. The absorbed mercury remains in the blood only for a short time but may remain in the tissues for a very long period and continue to exert its specific action by slow ionization and reabsorption.

Excretion. The excretion of this metal takes place mainly through the urine and fæces. Traces are also found in the bile, sweat and saliva; it is not excreted in milk. The form in which it is excreted in the urine is not known. In general, it may be said that with therapeutic doses excretion declines rapidly and becomes insignificant when the administration ceases. In the case of metallic mercury and colloidal mercury injections, absorption depots are maintained and traces may be excreted intermittently for as long as six months or even longer. A considerable portion of the absorbed mercury however is retained indefinitely in the tissues.

The fate of absorbed mercury and its distribution in the body tissues is not fully known. Its concentration in the blood, as has already been pointed out, declines rapidly and only minute traces can be detected in it afterwards. Its concentration in the urine apporximates to that in the blood. The kidneys usually contain the highest concentration and the largest amount. The liver comes next in order; in cases of intravenous injections of colloidal solutions and dyes, the concentration of the metal in this organ is especially high. The spleen, brains and lungs come next in order. The fat and the muscles contain a low concentration, but the quantity as a whole in the latter is fairly high. Mercury is found in the bones. In the body fluids and in the amniotic fluid mercury is found in fairly good concentrations. It passes through the placental circulation and hence it is claimed that congenital syphilis may be treated by mercurial inunction of the mother. Mercury in the spinal fluid increases in direct proportion to the number of doses, and the amount of the drug administered.

As antiseptic.

Mercury compounds were probably among the earliest antiseptics used, but it has now been recognized that their germicidal activity is not as potent as was supposed. The action is dependent on Hg ion which combines with all proteins, there being no selective action on the bacterial protoplasm. The inorganic salts of mercury have an irritant action and poor penetrability. They are fixed by proteins and lose their activity and are toxic. It has also been shown that their action is reversible as removal of mercury by precipitation from a supposedly sterile solution results bacterial growth. Spores are also very resistant to the action of inorganic compounds.

On account of these disadvantages a number of organic compounds of mercury were synthesized.

Mercury compounds.

(1) *Soluble.*—Perchloride of mercury (corrosive sublimate) is sold in tablets containing 0.125 and 0.5 gm. It is used in dilutions of 1 in 1.000 for sterilizing clothes, and 1 in 2,000 for sterilizing surgeons' hands. Other compounds are cyanide benzoate, salicylate and succinamide of mercury and red mercuric iodide.

(2) *Insoluble.*—Such as yellow mercuric oxide, calomel.

(3) *Complex organic combinations,* such as mercurochrome, metaphen, merthiolate, mersalyl, etc.

(2) Therapeutic uses of Mercury

As cathartic and intestinal antiseptic. Perchloride of mercury is seldom used internally as it is a strong irritant and corrosive and may give rise to toxic symptoms if the dosage is not properly controlled. In small doses however it is still used by clinicians for its antiseptic and astringent effects on the gastro-intestinal tract. Insoluble mercury salts are preferred for this purpose. Calomel dissolves so slowly and to so limited a degree that the intestinal irritation and toxic symptoms, commonly produced by the soluble compounds, are not manifested at all. On the other hand, a very mild irritation is produced which stimulates both the large and the small intestines and increases their peristaltic movements, resulting in catharsis. Ordinarily, calomel produces dark-green semisolid stools in ten to twelve hours. The green colour is due to biliverdin. The appearance of biliverdin in the stools has been considered to be due to the effect of calomel causing an increased flow of bile. This however is an erroneous idea. Numerous investigations with biliary fistulæ have shown that calomel does not increase the flow of bile.

Calomel is commonly used in therapeutics for its antiseptic action and it is one of the most useful and efficient antiseptics known. The antiseptic action is said to be due to the slow formation of mercuric chloride from calomel in the alkaline medium of the pancreatic juice. Following the administration of calomel, the bacterial flora is definitely diminished and this is reflected in the decrease of the urinary indoxyl. Large doses of calomel may favour bacterial growth by diminishing intestinal resistance.

Calomel is administered either in a single dose of 1 to 5 gr or more commonly in divided doses of ¼ gr every hour until 2 to 3 gr are given. The method of giving the drug in divided doses is more favoured, in view of the fact that this method ensures better solution and consequently more efficient action. Grey powder (hydrarg. cum creta) is specially suited for children. The mercurials are of value in obstinate constipation attended with biliousness and they can be conveniently administered at night followed by a saline purgative in the morning.

Calomel should not be used continuously for a long time on account of its systemic actions. This is ordinarily unimportant because any excess is generally excreted before it is absorbed. But conditions in the gastro-intestinal tract, *e.g.*, irritations, ulcerations, may favour rapid absorption and toxic symptoms. It is therefore always advisable to follow calomel after 6 hours by a saline purgative.

(i) Mercury in syphilis

Mercury has been in use in the treatment of syphilis from the 15th century and was the only drug having a definite anti-syphilitic action till the arsenicals came into use in the early part of 20th century. After the introduction of bismuth compounds it fell into background and now only used rarely. The reason for this is that mercury has a low therapeutic index against syphilis. Besides this, its action is very slow, and treponemes persist in the lesions because of delayed healing. It is doubtful if it has ever cured this disease during the centuries it has been in use. What it probably does is that because of its treponemostatic action, it reduces the infection to such a low level as to enable the body defences to cope with the infecting spironemes.

Mercury is undoubtedly inferior to arsenicals and bismuth compounds and even as an adjuvant to arsenicals, it is much inferior to bismuth in as much as it gives a higher incidence of relapses, and allows a lower number of serological

reversals. By itself, it does not prevent the infectiousness of the disease nor does it stop its progress. At the same time it is more toxic than bismuth and has a lower therapeutic index. Bismuth should, therefore be used when arsenicals are contraindicated and mercury should only be used under very special circumstances in early syphilis.

Mode of action of mercury in syphilis. The mechanism of antisyphilitic action produced by mercury is not understood. Though the metal has direct lethal effects on the treponema, the concentration necessary to produce such lethal effects is never reached in the blood in doses which do not exceed the tolerance of the patients.

Mode of action of mercury in syphilis.

(ii) Prophylactic uses

Mercury was used as a prophylactic remedy in syphilis for a long time. It often prevents the onset of primary and secondary lesions, if applied locally before the treponema have penetrated, *i.e.*, within 4 or at the most 8 hours after exposure. It has no preventive action 12 hours after exposure. The usual procedure recommended is to cleanse the site of the suspected infection with soap and dress the part with an ointment containing 33 per cent of calomel or 0.3 per cent of corrosive sublimate (Neisser-Seibert ointment). The prophylactics containing mercury do no real good but may do harm by preventing the recognition of infection.

Mercury still occupies an important position in the prophylaxis of syphilis, the preparation used being calomel ointment. Used properly within an hour after exposure it is said to prevent infection in 99 per cent of cases. Mercury ointments used are:—

U.S. army.—Mercurous chloride 30 per cent, benzoated lard 65 per cent, white wax 5 per cent. *U.S. Navy* Mercurous chloride 33 per cent, Camphor 2 per cent, phenol 30 per cent, anhydrous lanoline, 39 per cent, benzoated lard 20 per cent, bees wax 3 per cent. Prophylaxis also includes intra-urethral injections of argyrol 10 per cent or protargol 2 per cent solution by way of combined prophylaxis against syphilis and gonorrhœa.

As a diuretic. All mercury compounds during their excretion stimulate the renal epithelium and produce a distinct diuretic effect. This diuretic effect is particularly marked when there is œdema of the tissues. This property of mercury was known long ago and clinically made use of in the form of Guy's pill which is a combination of blue pill (pilula hydrargyri), with digitalis and squill. The diuretic effect of calomel was however unreliable and the diarrhœa set up during its administration was a very objectionable feature to the patients. Within recent years a number of stable organic mercury compounds have been prepared (see detailed description later on) which are soluble in water, practically non-irritant and non-toxic in therapeutic dosage, and can be used both intramuscularly and intravenously. Salyrgan (Mersalyl) and Novasurol (Mersaphen) are two well-known members of this group.

In cases of ascites following malaria and kala-azar, organic mercurial preparations have sometimes produced marvellous results. The excretion of fluid increases within 2 or 3 days of the administration of the drug and this increase is maintained for 6 to 7 days. These preparations have also been used in œdema following anæmia and hookworm disease. In the endemic ascites of the tropics, met with in Bengal and Bihar, novasurol and salyrgan have given good results.

Skin diseases. Mercury has been applied externally in various skin diseases of parasitic origin such as itch, ulcers, condylomata and ulcers of syphilitic origin. In all these conditions, mercury acts as a disinfectant and irritant. Ointments containing mercury in metallic form such as unguentum hydrargyri are the least irritant of all. Mercury oleate, yellow oxide, red oxide and ammoniated mercury are also commonly used. As a lotion, black wash or yellow wash is the common external application; as a dusting powder calomel may be used on ulcerating surfaces. *e.g.*, in corneal ulcers.

Gonorrhoea and gleet. Mercury has been used as a lavage in urethritis, a lotion of 1 in 4,000 to 1 in 2,000 of mercuric chloride being commonly employed.

(3) Modes of Administration

Mercury is administered chiefly by (1) inunction, (2) oral route, (3) intramuscular injection, and (4) intravenous injection.

Inunction. Mercury when triturated with fat forms a colloidal suspension which is absorbed when rubbed on the skin. Histological examination of the skin after inunction with mercury shows that the mercury globules do not enter the epidermis, but that they penetrate deep into the sweat glands and hair follicles and from there they are absorbed. It is a fairly effective method of administering mercury. Undesirable local and gastro-intestinal effects are not noticed and it is easy to avoid serious overdosage. The disadvantages are that it is time-consuming and disagreeable and the exact amount absorbed cannot be accurately determined.

For this purpose a strong mercurial ointment containing 50 per cent of metallic mercury is used but if it is irritating it may be diluted to 25 per cent with cold cream. The daily dose is 4.0 gm. Full instructions should be given to the patient how to use it, *i.e.*, where and how to rub it in. Inunctions should be discontinued if teeth start aching; generally there are no cumulative toxic effects. Various areas of skin should be used by rotation, as rubbing has to be continued for 6 to 10 weeks every night in the intervals between courses of arsenicals. Inunction is not used now.

Oral administration. The oral route is the easiest method of administering drugs but it is not suitable in the case of mercury and has now been largely replaced by other methods in adults. In the case of children, this method is still retained for the sake of convenience. Considerable quantities of mercury can be absorbed from the gastro-intestinal tract even when the insoluble mercurous compounds are administered. This is proved by the incidence of salivation and the urinary excretion which is quite as high as with inunctions and intramuscular injections. The gastro-intestinal irritation is the serious drawback which has restricted oral administration. The patient also cannot be kept under control and the rate of absorption cannot be determined with any degree of accuracy.

Mercury can be given orally either as a solution of a soluble mercury salt or as a pill containing an insoluble mercurous salt or as metallic mercury. The usual preparations used, in order of preference are *Hydrargyrum iodidum flavum* (Proto-iodide) dose 0.01 to 0.05 gm. (1/6 to 1 gr); *Hydrargyrum cum creta,* 0.05 to 0.2 gm (1 to 3 gr); *Hydrargyrum subchloridum,* 0.05 to 0.1 gm. *Hydrargyrum chloridum corrosivum,* 0.002 to 0.004 gm. (1/32 to 1/16 gr). To prevent local corrosion, mercuric chloride is administered with excess of potassium iodide or in milk.

Rectal administration, fumigation, inhalation of mercury vapourized at room temperature are no longer used in practice.

Intramuscular injections. Three classes of preparation are used for intramuscular injections: (1) water-soluble ionizable compounds, (2) water-soluble non-ionizable compounds, and (3) water-insoluble compounds.

Water-soluble ionizable compounds. Mercuric chloride (1 ccm of 1 per cent solution) injections are very painful and should not be used because pain and sloughing of tissues may follow, though some salts like the benzoate, succinimide, cyanide, biniodide (double salts) cause comparatively less pain than mercuric chloride in doses of 1.0 gm.

Basic mercuric salicylate is insoluble in water but is soluble in sodium chloride solutions or alkalis. It is given suspended in oil but is absorbed as rapidly as the water-soluble salts. It causes very little pain.

Water-soluble non-ionizing compounds. These include novasurol, salyrgan and mercurosol.. The antisyphilitic action of the first two is practically negligible. They cause very little local irritation and are not toxic in therapeutic dosage. The absorption is very rapid and fairly complete. These are used as diuretics and will be referred to again.

Water-insoluble compounds. These compounds are usually given suspended in oil. Although these are effective, they are now practically out of use. Their absorption is slow, since they must first be converted into soluble compounds; but when the injections are repeated, the absorption from the multiple depots becomes cumulative and uncontrollable. Stomatitis is a frequent complication. Calomel 10 per cent suspension in olive oil in dosage of ½ ccm weekly to 1 ccm twice weekly is used. Calomel has also been injected as a 5 per cent suspension with 2 per cent carbolic acid or creosote or camphoric acid. Metallic mercury, finely divided, as the 'gray oil' (oleum cinereum) has been injected in 40 per cent solution, ½ ccm weekly. Absorption is uncontrollable and toxic effects are common.

Intravenous injections. Intravenous injections of mercury were introduced with the idea of securing a maximal concentration of mercury in the blood without any of its unpleasant and untoward local actions. These however did not attain any degree of popularity. Preparations for intravenous injections are:

(1) *Ionizable salts.* Mercuric chloride, oxycyanide, benzoate and the double iodide have been administered in 1 per cent, solution in doses of 1 to 3 ccm daily or every second day.

(2) *Organic compounds.* Novasurol, salyrgan, mercurosol have been used to a much greater extent than the ionizable mercury salts. As has been already stated, these compounds are not very effective in syphilis and their chief use is in connection with the production of diuresis. With these compounds, as with ionizable salts, urinary excretion of the metal proceeds at a rapid rate within an hour or two, and declines promptly, reaching an insignificant level within 8 hours after injection. These therefore suffer from the same disqualification as the other group. Moreover, novasurol and mercurosol produce diarrhœa and dysentery-like symptoms in some patients. Salyrgan is not reported to have caused any untoward symptoms.

Disadvantages of intravenous administration. It will be seen that intravenous administration of mercury is not as effective therapeutically as the intramuscular method. Most of the compounds irritate the veins at the site of injection and tend to produce fibrosis and occlusion of the vein. They also present danger of embolism and colloido-clastic shock. Urinary excretion studies indicate that the high concentration reached by intravenous injection is of too brief a duration to be useful in chemotherapy and the maintained level is generally too low, even with daily injections.

(4) Toxic Effects

Acute toxic manifestations are produced, generally from mercuric chloride poisoning. The immediate effects are due to irritation, superficial corrosion and coagulation of the proteins of the mucous membranes. Salivation, swelling, burning and discoloration of the mucous membrane of the mouth and pharynx are constant features. Abdominal pain and distress with vomiting of bloody mucous shreds

are also met with. Twenty-four hours later, mercurial stomatitis develops but this is not very severe. Later, the large intestine and kidneys become involved. The urine becomes scanty and highly-coloured with copious albumin and casts. Anuria has occurred in some cases followed by death within a week. If the nephritis is not fatal. a membranous colitis develops with ulceration and hæmorrhage. Hepatic degeneration may supervene. Death occurs from circulatory failure in some cases.

Post-mortem examination shows the mucous membranes of the mouth, pharynx, glottis, œsophagus and stomach to be corroded and extremely congested. In the colon, necrosis and ulceration and consequent congestion are met with. The kidneys show signs of acute nephritis and the liver cells are degenerated in some cases.

The symptoms of acute mercurial poisoning are not produced by therapeutic doses of the drug and are therefore not very important from the clinical standpoint. A sort of subacute mercurial poisoning is the usual outcome of prolonged treatment. The symptoms which have to be guarded against by the physician in such cases are of a milder nature and consist of localised chronic inflammations, especially stomatitis, colitis, nephritis, etc. The stomach and large intestines are usually not involved as in the case of acute poisoning.

Stomatitis is usually the earliest symptom of subacute and chronic mercurial poisoning. It occurs constantly by whichever route mercury is administered. A metallic taste in the mouth, soreness of the gums and salivation are the primary manifestations. If not noticed in time and if the drug is pushed further, blackening of the gum margins, loosening of the teeth, uncontrollable salivation and later, fatal ulceration of the mouth may supervene. The kidneys are specially susceptible to mercury and therefore albuminuria is a common finding. The damage however is not as marked as in cases of acute poisoning and tends to involve the interstitial cells more than the glomeruli. Long continued exposure to relatively small doses leads to a slow and insidious development of chronic poisoning, usually with some stomatitis and renal irritation, but with additional nervous and nutritional disturbances. Cachexia with anæmia and malnutrition are found together with psychic irritability, tremors and restlessness.

Treatment of mercurial poisoning. The prognosis in cases of acute mercurial poisoning is grave unless prompt emesis is resorted to within 15 minutes of the administration of the poison. Mercury is rapidly fixed in the mucous membrane of the stomach and once it gets fixed, the antidotes (milk, calcium sulphide, thiosulphate, etc.) naturally cease to react. Lavage is sometimes helpful by removing the mercury which may remain free in the stomach at the time. The simplest antidote consists of three raw eggs in a quart of milk followed by gastric lavage. The local antidotes must be followed by an emetic to expel any poison which might remain unabsorbed. Administration of glucose and alkalies is useful and hot packs in the kidney region may help in soothing the renal irritation. Sodium thiosulphate has been advocated intravenously but is not very satisfactory.

In subacute cases, stomatitis and colitis are the symptoms to be attended to. Stomatitis is most effectively prevented and treated by hygiene and care of the teeth. Septic conditions about the gums are especially liable to cause poisoning and should be attended to before mercury treatment is started. When stomatitis is very severe, mercury should be stopped and mouth washes and gargles of an antiseptic nature should be prescribed. Hydrogen peroxide, potassium chlorate, potassium permanganate are all useful and should be used several times a day. The gastro-intestinal troubles usually yield to sedatives such as chalk and bismuth. Stimulants, whenever necessary, should be given.

Cases of chronic mercurial poisoning seen in workers in mercurial mines, etc., cannot be much improved even with vigorous treatment. In these cases, prevention and protection against unnecessary exposure are more important.

(5) Preparations of Mercury

The preparations of mercury which are recognised in the British Pharmacopœia are too well known to require any detailed description. In recent years attempts have been made to improve mercurial therapy and a number of organic compounds have been prepared.

Mercurochrome. Mercurochrome is disodium-dibromo-hydroxy mercury fluorescein. Dose 0.002 to 0.003 gm per kilo; in man 2 to 5 gr (0.13 to 0.32 gm). It is given intravenously in the form of 0.5 per cent solution in distilled water. Though solutions of 1.0 per cent and of higher strength have been recommended, they are not desirable. Mercurochrome 'sterules' containing 0.2 gm dissolved in 40 ccm of water for intravenous use are on the market.

Pharmacological action.

Pharmacological action. Mercurochrome is said to have a disinfectant action and is employed as a general antiseptic in surgery. A 2.5 per cent solution is useful for surface lesions, for painting on the mucous membranes or for injection into sinuses. Given intravenously in animals, mercurochrome causes no marked reaction if a 0.5 per cent solution is used. Stronger solutions produce a fall of blood pressure. Administration of 0.2 mgm per gm weight of mice produce immediate death of the animal. Small doses are therefore recommended in man and deaths have occurred with 8 to 15 ccm of a 1.0 per cent solution. It is absorbed into the blood and is excreted in the gastro-intestinal tract, in the bile and by the kidneys. It is, for this reason, used as an urinary and biliary antiseptic.

Therapeutic uses.

Therapeutic uses. Mercurochrome is recommended in all forms of bacterial diseases. It has been used in gonorrhœa, gonorrhœal arthritis, cystitis and pyelitis. As a vesical antiseptic, 1 per cent solution can be used for lavage without causing any pain. The routine method of giving vesical injections is to give 30 ccm of a 1 per cent solution; stronger solutions should be used with great caution. A 5 per cent solution is used as a uretheral lavage. Gonorrhœal arthritis is said to be favourable influenced by intravenous medication for which 10 ccm of a 1 per cent solution is employed. It has also been tried in plague, pneumonia, Malta fever, puerperal septicæmia, syphilis, typhoid and tuberculous affections. Mercurochrome has been tried in the treatment of intestinal protozoal infections in 1 to 5 gr (0.13 to 0.23 gm) doses three times a day, the drug being continued for 2 or 3 weeks. It is usually given in keratin-coated capsules and no harmful or distressing effects have been observed. Stools changes into a deep mahogany colour and must be kept thus so long as the drug is administered. Rarely, the drug produces intestinal cramps and nausea.

Mercurochrome has also been used in the form of colonic irrigations, a 5 per cent solution being injected slowly into the rectum in amœbic dysentery. In ulcerative colitis lavage with 6 to 8 ounces of a 0.3 per cent solution has given good results. It has been used against leprosy for checking rapid retrogression, in the treatment of ulcers following on disintegrating tubercles and in healing neurotropic ulcers; weekly injections of 1.0 per cent solutions are generally given combined with chaulmoogra oil esters.

Toxic effects.

Toxic effects. The toxicity of different samples varies. Salivation, stomatitis, and severe diarrhœa, commonly nausea and vomiting, rose-coloured stools, rigors and rise of temperature may occur after large doses. The kidneys may be irritated and albumin may occur in the urine. The margin between the therapeutic and toxic doses of mercurochrome is variable and small. As a rule no gastro-intestinal disturbances are produced till the drug has been continued for a week.

Flumerin. Flumerin is the disodium-hydroxy-mercuri-fluorescein. It is a red odourluess powder, somewhat hygroscopic, containing 33 per cent of mercury. It is soluble in hot water, about 1 in 10, but insoluble in alcohol, ether or chloroform.

Novasurol. Novasurol is also known as merbaphen; it is the double salt of sodium mercuri-chlorphenyl oxyacetate with diethyl barbituric acid (barbital). Novasurol occurs as a white crystalline powder, soluble in cold water and contains about 34 per cent. of mercury. It was originally intended to be an antisyphilitic remedy but has come to be used as a diuretic. When given intramuscularly it is excreted in the gut and doses higher than 2 ccm are liable to cause gastro-intestinal irritation with profuse watery stools.

The action of novasurol on the kidneys is important, since it has been used in cases of nephritis with œdema. The beneficial effect is more marked in œdema due to myocardial insufficiency than in cases of nephritis, where it is contraindicated. The exact mode in

which novasurol brings about diuresis is not known. In man, toxic symptoms have occurred after novasurol. In mild cases, certain disagreeable symptoms such as headache, vertigo, nausea, vomiting, stomatitis, diarrhœa, fever and rash are seen. These symptoms are generally mild and clear up with the discontinuance of the drug. In certain cases hæmaturia has occurred; death from the effect of novasurol is also on record.

Uses. It is useful in dropsies due to cardiac and cardio-renal diseases. It has been recommended in passive congestion of the kidney's, in nephrosis, and in arteriosclerotic and idiopathic contracted kidney. Ascites due to cirrhosis of the liver and Banti's disease is not usually benefited by the drug. As a diuretic 0.5 to 1 ccm of a 10 per cent solution is advised with simultaneous administration of ammonium chloride to improve the diuresis.

Salyrgan. This is another member of the organic mercury compounds which has been used in recent years very extensively in cardiac or renal œdema, to produce diuresis. It is a complex synthetic mercurial prepared by the action of mercury acetate and methyl alcohol on salicyl-allyl-amido-acetic acid and subsequent conversion to the sodium salt. The mercury content is about 40 per cent in non-ionizable form. It is a white crystalline powder, soluble 1 in 1 of water. It has been demonstrated to have spirochæticidal properties. The diuretic property of the drug is well known.

Salyrgan has been used in a great variety of conditions: (1) Cardio-renal œdema, (2) nephrosis, (3) malignant conditions with ascites, (4) cirrhosis of the liver, (5) tubercular peritonitis, (6) pleurisy with effusion, (7) malignant conditions with chylous pleural effusion. The advantages claimed for the drug are that stomatitis and proctitis, common with merbaphen, do not occur with salyrgan.

Metaphen. It contains about 58 to 60 per cent of mercury. Metaphen is a yellow substance, insoluble in water. For disinfectant purposes and for sterilising the skin solutions of 1 in 5,000 to 1 in 1,000 are prepared with the aid of caustic soda; for ophthalmic purposes solutions of 1 in 10,000 to 1 in 5,000 are preferred. Urethral lavage should be done with solutions of 1 in 10,000 to 1 in 5,000.

9. Iodides in Syphilis

For many years mercury and iodides were the only drugs available in the treatment of syphilis. The iodides have no direct destructive action on the spirochaetes. They, however, have a definite action on the granulomatous tissue such as is found in leprosy, actinomycosis, etc., and in some obscure way they act on the syphilitic lesions. According to some authorities they help the action of arsenic, bismuth and mercury, probably by increasing their penetrability into the syphlitic tissues in a way not yet understood. It is doubtful if iodides occur in such tissue in a higher concentration than plasma. They act on the syphilitic tissue even if they are not combined with other antisyphilitic drugs (arsenicals, etc.). They are said to inhibit the action of an antiferment and in this way the proteolytic enzyme is enabled to dissolve the necrotic tissue.

Value in syphilis. In early lesions iodides appear to have no marked action and arsenicals and bismuth compounds can produce their effects without their intervention. In late syphilis it is believed that if used in combination, the lesions are healed more expeditiously. They are particularly effective in gummatous lesions, cardio-vascular syphilis, syphilis of bones, skin, and meninges. In meningitis there is definitely an improvement in the symptoms. They should be given in large doses in all forms of gummatous lesions in combination with arsenic and bismuth. Iodides of sodium and potassium are effective, the other iodides having no particular advantages. They can be given in ordinary mixture with syrup or in milk to disguise their bitterish taste. In acute conditions such as meningitis as much as 15 to 20 grains have to be given three times a day if the patient can tolerate such doses. Formerly iodides were always combined with mercury but this is not the practice now and antisyphilitic treatment is given separately. In granulomatous conditions even larger doses are given. The drugs are best given by the mouth and before meals; they should be given with plenty of fluid.

Absorption and excretions:—Iodides are readily absorbed from the mucous membrane. They are absorbed from the stomach and are widely distributed throughout the tissues and body fluids in the same way as chlorides and bromides. They penetrate the red corpuscles but otherwise mostly remain extracellular in the body fluids. The view was formerly held that they are taken up selectively by the pathological tissues (granulation, tubercular etc.). They are completely excreted from the body in 24 hours; the excretion of the iodine ion is hastened by administration of large doses of chlorides. Iodides occur as HI, in gastric juice saliva, milk, bile, sweat, effusions etc.

Mode of action:—The iodine ions appear to have no specific action on any tissue in the body, nor even on the nervous tissues. Their action in syphilitic diseased tissues and in inflamatory conditions is not understood. It is well known that in tuberculous conditions they are contraindicated because they irritate and even lighten up dormant foci. Their action on gummata is probably of this nature which leads to their resolution.

Toxic effects:—Acute poisoning is not common unless the patient has idiosyncrasy (sensitiveness) for the drug. Before giving iodides by the intravenous route, the sensitivity should be tested. The chief danger is œdema of the larynx and consequent suffocation, and rarely multiple hæmorrhages in the skin and mucous membranes.

Iodism is the name given to chronic poisoning and this may suddenly develop when iodides are being taken. Individuals however, vary in the degree of their sensitivity. Sometime iodism is produced when small doses are given and disappears with larger doses, but it generally comes on when dosage is on the high side. There is a peculiar metallic taste in the mouth, burning sensation in the throat, soreness in gums and teeth, salivation, coryza, sneezing, irritation of the eyes, heaviness in the head or headache; in fact the symptoms resemble a cold in the head. There is increased secretion from the bronchii which in severe cases may lead to œdema of the lungs. Skin eruptions occur after prolonged use. Mental depression and occasionally severe diarrhœa and cachexia ensue.

Treatment:—In acute poisoning gastric lavage with starch solution or sodium thiosulphate solution (5 per cent) may be used to remove iodine. Normal saline is given intravenously. Iodism generally disappears a few days after the drug is stopped; excretion is hastened if large amounts of fluid and sodium chloride are given.

Preparations:—Besides the iodides of sodium and potassium, *Lugols' solution* which contains 5 per cent of iodine in 10 per cent solution of potassium iodide is used. A saturated solution of iodine is 15 grains (1.0 gm) per ccm. A number of organic iodides have been introduced which are less irritating but slower in action. Some of these Iodulbin (21.5 per cent iodine in blood albumin), Iodocasein (18 per cent iodine), are given in doses of 0.3 to 0.5 gm.

Lipiodol is a 40 per cent solution in poppy seed oil; dose 1.0 to 2.5 gm daily. It is introduced into the bronchi for taking X-ray pictures. *Riodine* is a 17 per cent solution in castor oil. *Diodrast,* a water soluble organic compound containing 49.8 per cent of iodine, is used intravenously for taking X-ray pictures of the urinary tract.

Iodides have been used empirically in all kinds of conditions such as hypertension, portal cirrhosis, encephalitis, aneurysm, etc. They are of real value in syphilis, hypothyroidism, as prophylactics in goitre, and as expectorants as they liquity tenacious secretions.

PART IV

REMEDIES USED AGAINST BACTERIAL DISEASES.

CHAPTER I

BACTERIAL DISEASES—SERA & VACCINES

GENERAL CONSIDERATION: BACTERIAL ORGANISMS, PATHOGENICITY OF ORGANISMS, RESISTANCE OF THE BODY AGAINST BACTERIAL INFECTION (IMMUNITY), MICROBIAL ANTAGONISM AND ITS BIOLOGICAL UTILIZATION—SPECIFIC IMMUNE THERAPY IN BACTERIAL AND VIRUS DISEASES—VACCINE THERAPY: PREPARATION OF VACCINES, VARIETIES OF VACCINES, METHODS OF ADMINISTRATION, THERAPEUTIC USES—SERUM THERAPY: MODES OF ADMINISTRATION, REACTIONS FOLLOWING ADMINISTRATION OF SERUM, ANTITOXIC AND ANTIBACTERIAL SERA, ANTIVIRAL SERA—BACTERIOPHAGE THERAPY: NATURE OR BACTERIOPHAGE, PREPARATION OF BACTERIOPHAGE, ROLE OF BACTERIOPHAGE, BACTERIOPHAGE IN PROPHYLAXIS AND TREATMENT OF DISEASE.

1. General Consideration

(1) Bacterial organisms

Bacteria are minute unicellular masses of protoplasm which are devoid of chlorophyll and definite nuclei. The dimensions of most of the cultivable forms of bacteria are of the order of low multiples or submultiples of a micron (μ) measuring 1/25,000 of an inch. These bacterial cells may be coccal or spheroidal, bacillary or cylindrical, or spirillar in shape. Many bacilli and most spirilla and vibrios are more or less actively motile by means of flagella. The most common method of multiplication of bacteria is by simple binary fission, each cell dividing into two daughter cells by constriction, these daughter cells again undergo similar division and so on. Throughout Nature bacteria are widely distributed. However, among the innumerable varieties of bacteria present in Nature there is a very limited group which is capable of becoming parasitic upon the bodies of higher animals, and among these a still smaller proportion which is capable of being 'pathogenic', *i.e.*, producing disease. The exposed parts of our bodies, the mucous membranes of the nose, the conjunctivae and the intestinal and respiratory tracts are constantly inhabited by a rich bacterial flora. Many of these consist of ordinarily harmless bacteria (saprophytes) and some are definitely beneficial and even essential for life.

The mere presence of any pathogenic organism in or on the body does not necessarily give rise to clinical disease. For the clinical manifestation of the disease to be produced it is essential that in addition to gaining access to the tissues the organism should be capable of maintaining itself and of multiplying in the tissues to a lesser or greater degree. There is a regular struggle between the invading organism and the defensive forces of the host. If the pathogenic organism gains a foothold in the body of the host, the infection is established and it is balance between the two opposing forces which decides the ultimate outcome of recovery or death. Before taking into consideration the diseases produced by the pathogenic bacteria it is, therefore, desirable to refer briefly to these two opposing forces, the capacity of the organism to cause infection, *i.e.*, its pathogenicity and the resistance of the body against bacterial infection.

(2) Pathogenicity of organisms

After an organism has gained a foot-hold in the body of the host its virulence or pathogenicity depends on its toxigenic or invasive powers or on a combination of both these properties. Some organisms, such as the *C. diphtheriae* and *Cl. tetani* produce their effects by the local production of a powerful toxin which diffuses into the tissues and produces its effects on cells which are far removed from its site of production. Others, such as *B. anthracis* and *Past. pestis* produce their pathogenic effects mainly by their power to invade the tissues and their main pathological effects are produced at the site or sites at which they are multiplying. Other organisms, such as *Str. pyogenes* are both toxigenic and invasive.

Bacterial toxins. The harmful effects of bacteria are dependent on the toxins they produce and on the constituents or products of bacterial cells such as congulases, fibrinolysins, substances increasing the permeability, etc., which, though not directly toxic, influence the course of bacterial infection. The toxins produced by the bacteria include filterable exotoxins which diffuse freely from site of infection and act specifically on certain sensitive cells; hæmolysins and leucocidins which act directly on red blood cells leucocytes and endotoxins which are contained in the bacterial cell and are liberated only when the cell disintegrates. *Bacterial toxins*

Relationship between virulence and dosage.—There is an inverse relationship between the virulence of the organism and the numbers or dosage which can bring about infection. In case of highly virulent organisms, such as the anthrax bacillus, the inoculation of a very small number of bacteria may suffice to initiate infection. It has been shown experimentally that a single anthrax bacillus may cause fatal infection in about 28 per cent of mice, while ten anthrax bacilli are capable of destroying all the infected mice. In contrast to this high virulence is the relatively low virulence of freshly isolated strains of meningococci or typhoid bacilli for mice. It appears to be quite reasonable to suppose that under natural conditions the spread of infectious diseases is conditioned to a great extent by the dose of the infecting agent. We can imagine that in highly infectious diseases, such as measles the amount of infecting material required to produce the disease is small while in diseases like whooping cough which are less readily contracted, a greater amount of inoculum is required.

The virulence of an organism is subject to great variations. It may be quite easily lost, for example by growing the bacteria on artificial media in the laboratory. Indeed, decrease in pathogenicity is almost the rule with most bacteria kept in the laboratory on ordinary artificial media. The virulence of such organisms may be restored by passage through an appropriate animal. By this way one can also frequently enhance the virulence of an organism for a species of host to which it is not naturally adapted. Variations in the virulence of the organisms may be of importance in deciding the characteristics of the various epidemics.

Channel of infection.—The path of entrance of an infecting organism is quite as important as its virulence. Even when an organism is pathogenic for the given animal, is virulent, and enters in adequate amounts, it may not produce the disease unless it enters the body by the particular path to which it has become pathogenically adapted. Thus, inoculation of the skin with virulent streptococci will cause progressive infection, but similar inoculation with the typhoid bacillus or the cholera vibrio will have no such effect. On the other hand while the swallowing of the typhoid bacillus will usually result in typical infection, swallow-

ing of pyogenic cocci will usually have no effect. Similarly, the virus of swine influenza produces the typical disease in swine only when inoculated by the intranasal route.

(3) Resistence of the body against bacterial infection.

So far we have discussed only the pathogenic powers of bacteria. The resistance of the host is the other factor to be considered as every infectious disease is the result of the struggle between these two opposing forces. Thus a micro-organism may be able to cause fatal infection in one individual but may be only slightly virulent or even quite harmless for another. Conversely, an individual may be highly susceptible to one micro-organism but resistant to others. Even the reactions of an individual to one and the same organism may differ at different times and under different circumstances.

Immunity

Immunity. The resistance or "immunity" of the host is of different kinds and of different degrees. The immunity may be innate or genetic or it may be acquired. Different species of animals are known to display varying degrees of innate or genetic (as opposed to acquired) resistance to the different pathogenic organisms. The acquired immunity is the result of natural infection or artificial immunisation. When acquired immunity follows infection or inoculation with vaccines or toxins of the pathogenic agent it is known as "active immunity" as the tissues of the infected or injected individual play an important part in bringing about the increased resistance. When acquired immunity is the result of transference of blood or serum of an actively immunised animal it is known as "passive immunity" since the tissues of the recipient play a relatively passive part. Topley and Wilson summarise this classification of immunity in the following table:—

1. Innate or genetic immunity
2. Acquired immunity
   - (a) Active
     - i. Naturally acquired
     - ii. Artificially induced
   - (b) Passive
     - i. Naturally acquired
     - ii. Artificially induced

Genetic and passive immunity.

Genetic and passive immunity.—About the innate or genetic immunity one may only say that the differences in resistance to a number of infections infecting certain animals seem to depend in part on some constant inheritable factors. Some of the differences in the racial resistances may belong to this category. The passively acquired immunity is the result of transference of ready made protective substances contained in the blood or serum of actively immunised animals. These substances may be transmitted naturally from the mother to her young, either by the placental circulation or in the colostrum. Artificial passive immunity consists in injecting the serum of actively immunised individuals or animals. The passive immunity is always short lived.

Active acquired immunity.

Active acquired immunity. The actively acquired immunity is the result of protective substances being formed by the tissues of the infected or injected individual. Active immunity is always of a longer duration than the passive and in case of certain diseases, such as measles, small pox, etc., the immunity may last for life. The protective substance or substances concerned in a specific

bacterial immunity are the antibodies which are prepared by the tissues of host in response to the stimulation produced by the "antigens" of the particular micro-organism. We now know that the essential agents in effective antibacterial immunity are those antibodies which act on the surface antigens of smooth, virulent bacilli.

Role of recticulo-endothelial system in immunity. The cells of the recticulo-endothelial system of the body play an essential part in the production of these antibodies. It will be remembered that these are the very cells which are responsible for removal of bacteria and particles of inert material from the blood. The recticulo-endothelial system includes a wide range of cellular elements which have a wide distribution in the body but all these cells function in the same manner like a single physiological unit. The spleen is the biggest of the single depot of these cells, the rest of the cells of this system are found in bone-marrow, liver, lymph glands and connective tissues.

Role of recticulo-endothelial system in immunity.

Now, we may refer briefly to the role of these antibodies in the antitoxic and the antibacterial immunity. The protection afforded by the anti-toxic sera depends almost entirely on the interception and neutralisation of the toxin before it reaches the susceptible cells. Once the toxin has reached the susceptible cells the anti-toxin is relatively ineffective. The neutralisation of the toxin by anti-toxin can be seen both in vitro and in vivo. The anti-bacterial immunity depends on increasing the efficiency of the clearing mechanism present in a normal individual. As a result the immunised animal deals with the virulent strain of an organism in the same way as a normal animal deals with an avirulent or slightly virulent strain of this organism. Of course, all this is specific, *i.e.*, this increase in efficiency of the clearing mechanism comes into play only for the particular organism against which the animal is immunised.

Non-specific factors. Non-specific factors occur in connection with body resistance. We have considered so far the specific resistance of the body to individual organisms. There is good ground for belief that any agency which interferes with the general health of the patient or which damages the tissues of the host will diminish natural or acquired resistance. Thus fatigue and chronic alcoholism cause a general deterioration of health; gross deficiency in intake of vitamins, especially vitamin A, markedly increases susceptibility to infection; injudicious diet predisposes to disturbances of the alimentary canal and increases the susceptibility to bacterial infection; trauma mechanically opens a road into the tissues and creates a nidus for the growth and multiplication of the invading organisms; fluctuations in temperature and humidity affect the local condition in the upper respiratory and the gastro-duodenal tracts and may lower the local bactericidal mechanisms. Seasonal variations in the course of infectious diseases and in their frequency are quite common, it may be that seasonal variation affects the resistance of the host, as well as the virulence of the parasite.

Non-specific factors.

Local Immunity. The mechanism of specific immunity that we have discussed above depends on the presence of circulating antibodies which interacting with the pathogenic bacteria and their products either kill them directly or neutralise the toxin and prepares them for ingestion by the phagocytic cells. Since these antibodies are distributed throughout the system the resulting immunity is a generalised one. There is however, some evidence to show that under certain circumstances immunity localised in certain areas of the body may exist in the absence of a general immunity. The work on experimental erysipelas in rabbits has shown that following intracutaneous injection with living streptococci local

areas of skin may be more resistant than the general skin to further intracutaneous injections of these organisms. It has been found that after repeated inoculations there is generalised skin immunity and that this is associated with appearance of specific antibodies in the blood.

This local increase in resistance has not been found to be of specific nature and local non-specific inflamatory reactions following injection of substances like mustard oil and sterile broth, etc., are capable of producing non-specific local immunity. Histological studies show that this non-specific increase in local resistance is determined to a great extent by mobilisation of various phagocytic cells. Thus there is no doubt that local immunity as opposed to general immunity does exist, but the increased local resistance is largely determined by non-specific factors, although the possibility of specific factors being involved can not be excluded.

Tissue immunity.

Tissue immunity.—The problem of the existence of local immunity as opposed to general immunity is quite distinct from, although it is often confused with the problem of the existence of tissue or cellular immunity as opposed to 'humoral' immunity. The propounders of the theory of 'tissue immunity' believe that local tissue immunity as such, and independently of associated humoral immunity, confers general immunity on the animal. Besredka (1919, 1920, 1921) has been the chief exponent of this view. Starting with the observations regarding the specific localisation of many pathogenic bacteria in particular tissues of the body, Besredka reasoned, that these particular tissues are the only tissues susceptible to the particular organism, and that if these tissues are immunised the whole individual will be immunised. According to this theory it is only the cells in the skin that are susceptible to anthrax bacillus, and only the cells in the intestine that are susceptible to typhoid, para-typhoid and dysentery bacilli. The immunity to infection of an immunised animal, according to his theory, depends on the immunity conferred on the susceptible cells of a particular tissue. For example, the anthrax bacillus when it reaches the skin encounters susceptible or "receptive" cells with which it reacts and produces substances that inhibit the activity of the phagocytes. If it reaches the body through some other route, which avoids contact with the skin, no 'receptive' cells are encountered and no disease results since the organism is rapidly destroyed by the phagocytes. According to this theory immunisation would consist in rendering the 'receptive' cells resistant by desensitizing them with products derived from the particular bacteria. The experimental evidence on which Besredka based his theory has been challenged. His statement that after oral administration of dysentery or typhoid bacilli the agglutinins may appear in the blood of rabbits after the first administration, but they soon disappear, and do not reappear after any subsequent administration, has not been confirmed. There has been good deal of controversy and conflicing experimental evidence regarding his statement that the skin alone was susceptible to anthrax infection; while some workers have confirmed Besredka's findings, most have failed to do so. It has been estimated that the minimum lethal dose of a culture of anthrax bacillus was much less when injected intramuscularly than intracutaneously. Cultures of anthrax bacillus in candles sealed in the peritoneal cavities of nine rabbits produced death in seven after intervals varying from 6 to 51 days. Burke and Barnes (1931) succeeded in producing initial anthrax infection in the subcutaneous tissue, they utilised the bactericidal action of gentian violet to make sure that the skin was not infected at the time of the subcutaneous injection.

Oral administration of vaccines.

Oral administration of typhoid vaccine.

Oral administration of typhoid vaccine. Besredka has applied his theory to immunise human beings against typhoid by oral administration of typhoid vaccine and to treat staphylococcus and streptococcus infections of the skin by local

application of dressings saturated with filtrates of cultures of these organisms. There is evidence that the oral administration of typhoid vaccines does have some immunising value, and we know that oral administration of living or dead bacteria is often followed by passage of antigen of the bacteria through the intestines, and by the consequent production of antibodies. The oral administration of vaccines is a less reliable and quantitatively very uncontrollable substitute for subcutaneous injection. Regarding the local application of the so-called 'anti-virus' preparations in the treatment of staphylococcal and streptococcal skin condition it would appear that the result is entirely non-specific since infiltraton of the skin even with sterile broth raises the local resistance.

(4) Microbial antagonism and its biological utilization

Bacteria causing cholera, dysentery, typhoid, etc., enter the soil but do not survive there for long. It was suggested that the cause of this disappearance is to be looked for among the soil inhabiting microbes antagonistic to the pathogens. Harvey induced a typhoid carrier to urinate on soil and towels. After 30 hours the organism could not be recovered from the soil that was still moist, but were recovered from the towel that had been allowed to dry. Rhines showed that *Myco. tuberculosis* multiplied in sterile soil, but in non-sterile soil it was destroyed. Frost proved that saprophytes in the soil produce substances that not only inhibit the growth of pathogens but bring about their destruction.

The antagonist organisms described so far can be divided into four groups. The first group comprises various strains of *Ps. aeruginosa* and *Ps. fluorescens.* The active substance according to Hettche is of a lipoid nature; various workers have reported it to be active on certain gram-positive and gram-negative organisms.

The second group comprises various spore-bearing bacteria of *B. mycoides* and *B. mesentericus* group. These bring about lysis of diphtheria, typhoid and cholera organisms. They are highly specific in their action. Dubos has isolated 'Gramicidin' from a soil bacillus that protects white mice against infection with large numbers of virulent pneumococci. It also exerts a curative effect when administered to mice several hours after injection of pneumococci. Encouraging results have also been obtained in treating bovine mastitis with this substance.

In the third group of antagonists there are certain actinomycetes. Waksman and Woodruff have isolated a highly active substance that inhibits the growth of *S. lutea, Bac. mycoides, B. abortus* and *E. coli* in very high dilutions.

The fourth group consists of antibiotics derived from fungi *e.g.* penicillin.

The utilization of the antagonisitic activity of various soil microbes opens a new field for the preparation of various chemotherapeutic agents, for combating human, animal, and plant diseases, and the extraction of these antibacterial or anti-toxic principles, and determining their nature and mechanism of their action can lead to the development of chemotherapy on a rational basis.

2. Specific Immune Therapy in Bacterial and Virus Diseases

In the prophylaxis and treatment of a large number of bacterial and virus diseases specific immune therapy has been employed. With the introduction of sulphonamides and antibiotics the use of sera and vaccines in the treatment of many infectious diseases has become of secondary importance. Both these groups of therapeutic agents have powerful bacteriostatic and bactericidal properties. They do not, however, neutralise the toxins produced by the infecting organisms. Sera are, therefore, used in combination with them whenever it is desired to

neutralize the toxins. Vaccines have a limited application in the treatment of disease, but their prophylactic value in a number of infectious diseases is a great asset. The specific immune therapy may either be active or passive. The object of the active immunisation is the specific stimulation of the natural immunity mechanism of the body through the inoculation of a suitable suspension of the causative agent of the disease, *vaccine therapy*. Passive immunity aims at the destruction of the infecting organism or the neutralisation of its toxins, through the inoculation of ready-made specific substances or antibodies which are present in the sera of immunised animals, *serum therapy*. These two measures are suited for different types of diseases and when appropriately used they produce favourable therapeutic results. Serum therapy has been found invaluable in certain acute infections, where a rapid neutralisation of the poison is indicated and where it is desired to aid the overtaxed immunity mechanism of the patient. Vaccine therapy on the other hand has been of benefit in prophylaxis and in treatment of a number of chronic localised infections, where the patient is not too ill and his immunity mechanism is not exhausted. But unfortunately at the present time, because of the growing tendency to apply these remedies in all sorts of unsuitable conditions, both forms of specific immune therapy in general and vaccine therapy in particular , have fallen into disrepute. In many instances the practitioner employs vaccine therapy not because he considers it suitable but because other remedies have failed and he cannot think of anything better. The common excuse is that "if vaccines do no good, at least they do no harm." This appears illogical, as we believe that any remedy that has potentialities for doing good also possesses the power to do harm, if improperly used. The unscientific attitude, the commercial exploitation of vaccine therapy, and the utter disregard of the results which observation and experiment have made available, are to be greatly deprecated. The improper application of the therapy will only retard the progress of science and will help neither patient nor practitioner. It is the clear duty of the medical practitioner, by study and knowledge of the latest advances in immunology and bacteriology, and through the proper and scientific application of these valuable therapeutic agents, to contribute towards the progress of science and thus to the ultimate well-being of the community. The best thing for the practitioner to do is to use only those immunising reagents which have been shown to possess definite protective value under adequately controlled and extensive trials.

3. Vaccine Therapy

The word vaccine is from the latin *vacca,* a cow. Cow-pox was called *vaccinia* or cow-disease and Jenner designated protective inoculation against small-pox with cow-pox virus as *vaccination.* Pasteur adhered to Jenner's nomenclature and applied the term *vaccine* to the suspensions of attenuated and killed bacteria that he used for immunisation. At present the term *vaccine* may be defined as a suspension of killed or attenuated organisms or their products, which when inoculated into the body, stimulates the tissues to produce antibodies which directly or indirectly bring about the destruction of the invading organisms and the neutralisation of the poison that the organisms may have produced.

Vaccine therapy has been in practice for a considerable length of time. In ancient China and India, the people had observed that one attack of small-pox protected them from subesequent attacks. They put this observation to practial use by exposing their children to mild cases of small-pox in order that a mild infection might be acquired and a lasting immunity secured. This practice was, of course, crude and attended by grave risks as it sometimes resulted in virulent infection and death. Many centuries later, Edward Jenner, as a result of his own observations and experiments, proved that cow-pox virus when inoculated into human beings produced a trivial infection followed by an absolute immunity

against small-pox. Although he could not explain the mechanism involved in the process of immunisation, he may be justly considered to have laid the foundations of prophylactic vaccination. After Jenner, came Pasteur who while working with the organism of chicken cholera found that by leaving virulent cultures of the organism at the laboratory temperature for some weeks they became innocuous to chickens and were capable, when inoculated, of producing immunity in them. These inoculated fowls proved resistant to a subsequent inoculation with virulent organisms. From this he conceived the idea that by attenuating virulent organisms, and inoculating them into animals immunity might be produced. He put his theory into practice in a number of other bacterial infections and obtained convincing results. He established the value of prophylactic vaccination beyond any doubt. Following upon Pasteur's discoveries, preventive inoculation began to be widely practised both in animals and in man. In all his vaccines, Pasteur used attenuated living virus. The use of such vaccine in human subjects was attended with a certain amount of risk.

Salmon and Smith (1884-86) were the first to show that injection of dead cultures of bacteria were capable of establishing immunity in animals. But it was not till the work of Haffkine, Pfeiffer, Kolle and Wright, several years later, that the modern method of prophylactic vaccination in man was well established. Wright and his colleagues advocated its use for curative purposes, and according to them the justification for curative vaccination lay in the fact 'that in many instances infections are localised and that, while the local capacities of resistance may have been lowered, the immunity mechanism in other parts of the body may not have been brought into play and that vaccines by stimulating these may flood the focus of infection with antibodies and phagocytes and overpower the causative agent.' The experience gained during the past fifty years has vindicated in a convincing manner the value of vaccine therapy specially in the prophylaxis of the disease.

A considerable amount of controversy has taken place as to the comparative value of living attenuated vaccine and vaccines prepared from killed cultures. Considering that a certain amount of risk is attended with the use of living organisms for this purpose, the general opinion is in favour of the use of vaccines prepared from killed cultures.

(1) Preparation of vaccines

The selection of strains for the preparation of vaccines is of paramount importance in view of recent knowledge of bacterial variation and the associated changes in antigenic structure of the bacteria. It is most essential to use a strain containing antigens which can be reasonably expected to confer active immunity against the particular infective disease. From this point of view two types of variations are of great significance. One is the 'S' & 'R' variation and the other the 'H' & 'O' variation.

Arkwright (1927) working with typhoid--paratyphoid-dysentery group of bacteria described the occurrence of 'rough' colonies in cultures of those organisms which normally give rise to 'smooth' shiny colonies. This change from 'smooth' to 'rough' growth is associated with a marked change in antigenic structure. The normal or the 'smooth' form is known as the 'S' form and the rough variant is known as the 'R' form. The 'R' variant is characterised by loss of normal type specific antigen and is associated with loss of virulence. This 'S' & 'R' variation has now been shown to occur in various other bacteria. As the 'R' variants are wanting in the essential antigen it is quite apparent that they are worthless as far as the production of protective vaccines is concerned. In view of this it is essential that in the preparation of vaccines 'R' variants should be rigidly excluded.

Smith and Reagh (1903) demonstrated the difference in the agglutination reactions between motile and non-motile forms of the same organism and later Weil and Felix (1916) working on the agglutination of *Proteus vulgaris,* differentiated two types of colonies in the culture; the one is of a spreading type and the other discrete. The former is known as 'H' variant and the latter 'O' variant. It has been shown that the 'O' antigen of the smooth type of organisms is the one which is most important in the production of immunity and the 'H' antigen and 'R' somatic antigen are of no protective value. In addition to the 'O' or the somatic antigen, and the 'H' or the flagellar antigen some bacteria have been shown to possess a Vi antigen responsible for virulence. It has been demonstrated that degree of protection afforded by typhoid vaccine is closely related to the virulence of the culture used to prepare it, virulence has been correlated with Vi antigen. Typhoid cultures freshly isolated from blood invariably contain Vi antigen, but gradually loose it on subculture on ordinary media. It is advisable, therefore, to select a freshly isolated smooth strain containing O antigen as well as being particularly rich in Vi antigen.

Medium. Solid medium is better than liquid medium, it yields more growth and no constituents of the medium are included. In liquid medium autolysates of the organisms which are generally toxic and produce reaction are generally included. Enrichment of the medium is permissible but no horse serum should be added to avoid serum sensitivity.

In diseases like small-pox and rabies the causative agent (filtrable virus) cannot be grown in culture media. Therefore to prepare vaccines of these viruses they are cultivated in the tissues of animals (on the shaved skin of the abdomen of healthy calves in the case of small-pox virus and in the brain of sheep in the case of rabies virus) and an emulsion of the infected tissues made and tested for purity bacteriologically. The preparation and standardisation of these vaccines require great care and technical skill and in some countries a license has to be obtained for preparing these and only well equipped laboratories of a first class nature are granted permission to manufacture them.

Many of the viruses can be grown in chick's embryo and vaccines for prophylactic purposes have been prepared. For the preparation of vaccines which consist of the exotoxin or endotoxin of organisms, as for example, Koch's tuberculin, diphtheria, tetanus etc. the organisms are grown in special fluid media and when the maximum degree of toxicity is reached the medium is filtered, the bacterium-free filtrate standardised and vaccine prepared from it.

Time of growth before harvesting. For economy try to get as much growth as possible, but as a general rule incubation should be for 18 to 24 hours. In prolonged incubation autolysis comes in, which is not desired and in certain cases the organism changes, *e.g.*, in mucoid streptococci the capsule disappears after sometime.

Maintenance of strain. The strain properly identified and selected should be maintained on a suitable medium. The stock might also be maintained in the dry state. In the case of the typhoid bacillus occasional mouse passage is recommended. Every time before the preparation of a vaccine from a stock culture, after a preliminary transfer, identification and purity tests should be repeated.

Harvesting and *suspension.* Add saline to each bottle, set for a while, then by rocking most of the growth will be washed off. A stainless steel rod terminating in a triangular loop, or some other such gadget can be used for scraping. Be careful to just scrape only the growth and not to break the surface of the medium or get any particles of it in the suspension.

Washing bacterial vaccine. Any material from the media should be got rid of. Autolysis products are removed by washing and only the cells are left; sometimes flagella and capsule may also be lost. The product looks better and there are few complaints of reactions. This procedure is not followed everywhere.

Enumeration. In principle, the methods used for enumeration may be classified as those of:—

(1) Direct enumeration in a counting chamber.

(2) Indirect enumeration:
   - (a) Wright's method.
   - (b) Colony count, by making pour plates of differnt dilutions of the suspension.

(3) Physical measurements:
   - (a) Weight of dried bacteria.
   - (b) Volume of bacteria.

(4) Chemical measurement:
   - (a) Total nitrogen determination of the suspension; micro-Kjeldehal method recomended by Mueller is the best.

(5) Turbidity measurements:
   - (a) Opacimetric.
   - (b) Nephelometric.
   - (c) Photometric.
   - (d) Photo-electric.

No matter what method is adopted for standardizing vaccine the result is expressed in millions of bacteria per ccm. The counts of organisms fail to take into consideration the size of the organism, therefore, the antigenic content will be better represented by the volume, or the dried weight of bacteria. As antigens are closely associated with proteins,

the amount of total nitrogen of the bacteria might be an even better measure of the amount of antigen present. Until there is some general agreement, it is better, for the sake of uniformity to express the bacterial content in numbers. The most popular methods at present, because of their simplicity and speed, are turbidity methods. Gates apparatus or its modification are used in various places; or comparisons are made with standard suspensions such as Brown's opacity tubes. Bayliss has proposed a photometric method, and recently photoelectric cell has been utilized for determining turbidities of bacterial suspensions.

Standardization. The antigenic value can be measured by protection test in mice, or agglutinin production in rabbits and man. Typhoid is a good example for illustration: 0.5 ccm of regular typhoid vaccine injection, after 10 days will protect a mice against a lethal dose of typhoid culture; 10 organisms of typhoid or para-typhoid B with mucin are lethal for mice. A vaccine of low protective value cannot be expected to be of much use.

In rabbits living Vi bacilli produce Vi agglutinin titre that runs approximately parallel with the protective power against living virulent bacilli, and the O agglutinin titre runs parallel with the protective power against the toxic action of killed typhoid bacilli. The protective power of a vaccine can be judged by its capacity to induce the formation of Vi and O agglutinins in rabbits and finally in man. This is simpler than protection experiment in mice. The popular use of these methods is strongly suggested.

Killing and preserving. Heat is mostly used for killing, and chemicals for preserving. When heat is applied, it should be the lowest temperature at which one is sure to kill the organism. Generally 53°C for 70 minutes is quite enough. Amongst the chemicals, phenol, formalin, mercurials, tricresol, and alcohol, etc., have been used. Recently for typhoid vaccine Felix has recommended killing the bacteria with 75 per cent alcohol, removing it and preserving it in 25 per cent alcohol in saline. In his opinion alcohol is the best preservative for Vi antigen.

The last step is to test for sterility before dispensing. Most of the vaccines if kept under proper conditions are considered satisfactory for use for 9 months to a year from the date of preparation.

In 1908 Calmette isolated a strain of bovine tuberculosis which has been cultured since then on a medium consisting of glycerine, bile, and potato. This strain appears to have largely lost its capacity to produce progressive disease in all animals, but is supposed to be capable of producing immunity. Vaccine prepared from this strain is known as B. C. G. (bacille Calmette-Guerin). Calmette reported that after the administration of 50 mg of this vaccine subcutaneously, calves become resistant to doses of virulent bovine bacilli which would kill normal animals within two months. Innumerable trials have been made with B. C. G. in animals and human beings. Irvine had reviewed the literature up to 1934. He concluded that the virulence of B.C.G. is not fixed, although it had not been proved to have caused disease in man, it might rarely produce progressive tuberculosis in animals. As far as its value as an immunizing agent is concerned, it produced in cattle a definite degree of immunity, and in man it semed to have increased the resistance, but not so clearly. Park and Kereszturi in America are of opinion that the virulence of B.C.G. by residence in the human body is not increased and their opinion is shared by the majority. Their data indicates that resistance to tuberculosis is considerably increased and they advocate its use in possible contacts who are not yet infected, like new born children in a family with a tubercular member. Others are against its use because of the troublesome small cold abcesses that may persist for months at the site of inoculation. There is also chance, however remote, of its becoming more virulent. Plahiff recently tried a heat-killed vaccine in Jamaica. The mortality in non-vaccinated was 9.9 per cent and among the vaccinated 3.7 per cent. In view of these results with killed vaccine we might conclude that B.C.G. definitely increases the resistance to a moderate degree but is not decisively superior to killed vaccines. The protective value of B. C. G. in nontuberculin reactors has in recent years been established beyond all reasonable doubt as a result of millions of vaccinations all over Europe and America.

(2) Varieties of vaccines

'Live' vaccines.—As the name implies organism in this vaccine are alive and not dead. Theoretically the ideal form of vaccine is the 'live' vaccine, because there is experimental proof that it calls forth a maximum response of antibody. In the prevention of small-pox the marvellous results obtained are due partly to the fact that the vaccine used is a 'live' vaccine. Recently, Alcock has used 'live' vaccine in gonorrhœa, but his results are not encouraging. While there are advantages in using a 'live' vaccine. the procedure is attended with grave risks and, therefore, cannot be recommended as safe for employment in human cases, except in a few select instances.

Sensitised vaccines. These were originally introduced by Besredka, and are prepared by growing the organism in suitable media and then mixing it with the appropriate immune serum, allowing the specific antibodies to get absorbed on to the organism, centrifugalising the suspension and finally resuspending the sensitised organism in normal saline. The vaccine is really a sero-vaccine and has antibodies present in it along with the organism. By sensitising the organism, the therapeutic value of the vaccine is said to be enhanced because larger doses can be given and there is very little reaction after injection. The preparation of sensitised vaccine is not only difficult and expensive but it is also not possible for all organisms. It can be made only in those cases where a potent specific anti-serum is available. It is commonly used in streptococcal infections and is well tolerated even by patients suffering from acute and generalised infections.

Autogenous vaccines. These are vaccines prepared from the organism causing the infection in the patient. Here one is certain that the vaccine prepared is antigenically specific and will give rise to antibodies that will produce maximum good to the patient. Wherever possible autogenous vaccine made by a competent bacteriologist should be used.

Formolized vaccines have been used in cases where the culture of the organism produces a powerful toxin. It has been shown that toxins treated with formalin lose the toxic property but retain the antigenic property. This principle has been applied to whole cultures. Broth cultures or saline suspensions of organisms are incubated with 0.2 to 0.5 per cent. formalin which procedure kills the bacteria, and where exotoxin exists, converts it into a nonpoisonous toxoid but still retaining its antigenic properties. It has been used with success in the case of Shiga's dysentery bacillus. The formol toxoid now so largely used for immunisation in diphtheria is produced by incubating a toxic filtrate with 0.4 per cent formalin for several weeks.

Defatted vaccines. Douglas and Fleming (1921) claimed that a tryptic digest of acetone-extracted bacteria furnished a good antigen. Dreyer (1924) prepared tubercle vaccine by first boiling the organisms in formalin and then extracting them with acetone. He claimed to have cured tuberculosis in guinea-pigs with the use of such a vaccine. Clinical trial in man appeared to be favourable in the beginning, but later observation did not show any advantage of this type of vaccine over the ordinary vaccines.

Lipo-vaccines. In these the bacteria are suspended in an oily medium, so that the vaccine substance would be slowly absorbed and the antibody-producing stimulus would be prolonged, thus approaching more nearly the condition in an actual infection. The available evidence goes to show that these are less effective than the ordinary vaccine suspended in saline solution.

Vaccines killed by chemical substances. Vaccines killed with various chemicals such as ether, iodine, and sodium fluoride have been extensively used in France with the idea that these not only kill the vegetative forms of bacteria but also detoxicate them, but they have not found favour anywhere else.

Bacterial extracts, filtrates and digests. With the idea that antigen in solution can react immediately with the body cells thus enhancing the immunisation process such extracts have been used. Although these are antigenic there is as yet no evidence to show that they are superior or even equal to the simple bacterial vaccine.

Autolysates. Sometimes the breaking down process proceeds too far and the immunising power of the vaccine is lost. More work is needed in this direction before this type of vaccine can be advocated for general use. In this connection it may be stated that bacteriophage is really a combination of bacteriophage and vaccine.

Antiviruses. These were introduced by Besredka (1919-24) with the idea that filtrates of cultures which have stopped growing contain inhibitory substances to which he gave the name 'antivirus.' He claims that animals inoculated subcutaneously or intradermally or poulticed on their shaved skin with staphylococcal or streptococcal antiviruses are resistant to inoculation of virulent cultures introduced 24 to 48 hours later. But this has not been substantiated by other workers.

(3) Methods of administration

Vaccines are administered by the following routes:—

Subcutaneous.—This is the route commonly chosen. Vaccines are best given at a point where the tissues are loose. The popular site is the outer aspect of the arm about one-third of the distance down from the shoulder to the elbow.

When large quantities have to be given as in antirabic treatment the sides of the abdomen may be preferable. In the case of localised lesions some believe that injection of the vaccine near the lesion is more advantageous, but there is no evidence in support of it.

Intravenous. Vaccines are sometimes given intravenously for the purpose of eliciting marked reactions. In the treatment of arthritis, typhoid, coli and gonococcal infections vaccines have been given in this way. The beneficial results noticed appear to be due to non-specific protein reaction that follows. This route is not however satisfactory for routine use due to presence of particulate matter in vaccines and due to the danger of producing protein shock.

Oral vaccines have been given by the oral route specially in little children and in patients who cannot overcome their antipathy to inoculations. Besredka's bili-vaccines are prepared specially for oral administration.

When vaccines are given orally there may be certain amount of absorption of antigens through the intestinal mucosa with the consequent production of a certain amount of antibodies. But the immunity thus produced can never be expected to reach the level attained after say a subcutaneous injection and it is doubtful if it is efficacious at all.

Intracutaneous. Small-pox vaccine is used in this way. The cleaned skin is scratched gently by a needle or lancet, care being taken not to draw blood and then vaccine lymph is applied to the scarified part and allowed to dry. Some prefer to give bacterial vaccines also by this route, but the advantages claimed are of doubtful value.

Dosage. Dosage varies not only with the nature of the vaccine used but also with factors such as age of the patient, nature of the illness, toxicity of the organisms, sensitiveness of the tissue affected, route of administration and the purpose of administration. In young children, in acute illness, when sensitive tissues like the lungs or the brain are involved, when the selected route of administration is intravenous or the purpose for which it is used is for cure of illness, the dosage has necessarily to be small. On the other hand, in adults, in chronic illness of a localised character, when the route is subcutaneous or when the purpose is prophylaxis, a larger dose will not be harmful. The first dose is purely an experimental dose, and the subsequent doses must be controlled by the local, focal and general symptoms. The production of any marked reaction, either local or constitutional, after an injection may be considered a contraindication to any increase in dosage. The time interval is another important factor to be considered. As a rule, 3 to 5 days or 7 days' interval between doses is given for chronic or subacute infections and 48 to 72 hours' interval in acute cases, but there is no hard and fast rule and the reaction of the patient is the best guide. When stock vaccines are used the dose may be two or three times as large as those of autogenous vaccine and when used for prophylactic purposes they may be about five times the initial dose. For assessing the importance and value of the different factors discussed, experience alone is the chief guide.

Reaction following administration.—Local, focal and general reactions may be noticed after vaccine administration. The first is characterised by pain, swelling, redness and heat at the site of inoculation, the second by exacerbation of the symptoms due to the lesion and the third by slight chill, rise of temperature to 100 or 101°F, nausea, and headache. These symptoms usually subside within 24 hours. Neither of these reactions is generally present if the dosage is carefully chosen. It is advisable, in the best interest of the patient to avoid these constitutional disturbances because the local and general reaction play no part in the production of immunity. The initial dose being a trial one, there may occur some slight focal reaction, but this should not be allowed to recur. Focal reaction is of a mild nature and disappears spontaneously.

(4) Therapeutic uses

The most convincing results have been obtained by the use of vaccine for prophylactic purposes. There is little doubt about the efficacy of preventive inoculation in small-pox, rabies, diphtheria, scarlet fever, cholera, typhoid fever, and plague. As regards diseases such as influenza, whooping cough and typhus,

the results are encouraging. On the other hand the therapeutic use of vaccine has not been very successful so far, but lately they have been used more extensively with greater success on account of the better method of preparation. The success depends not only on the proper preparation of vaccines but also on the proper choice of cases. When a course of an autogenous vaccine fails to produce the desired effect, attempts should be made to detect any fresh infection by some other organism or by a different serological type of the same organism. In either case a fresh autogenous vaccine should be prepared incorporating the new organism. Sometimes it is difficult to isolate any organism from an infective focus or those isolated are of no use as vaccines. In these cases, the best results are obtained with stock vaccines. The advantages of autogenous and stock vaccines have already been discussed.

In some cases it is not enough to stimulate the defence mechanism of the body by vaccines alone but steps should be taken to ensure phagocytosis and the access of antibodies to the foci of infection. For example in the case of chronic abscesses vaccines by themselves will do no good unless the abscess is opened and evacuated as well. It cannot be overemphasized that a properly prepared vaccine should be considered as a potent substance capable of causing much harm to the patient when wrongly used. Before the administration of a vaccine all these points should be carefully considered. In competent hands results are very encouraging in cases of localised infections and evidence is gradually accumulating to show that vaccine may also do good in generalised infections as well.

The following is a brief outline of the more important vaccines used either in prophylaxis or treatment of diseases.

Typhoid Vaccine. This is a mixed vaccine consisting of a carbolised saline suspension of three organisms, *Bact. typhosum* 1,000 millions, *Bact, paratyphosum A* 500 millions and *Bact. paratyphosum B* 500 millions per ccm. Felix has recently recommended an alcohol killed and alcohol preserved vaccine which retains the Vi antigen and produces better immunity.

For prophylaxis it is given subcutaneously in two doses of 0.5 ccm and 1 ccm at an interval of 7 to 10 days. Some prefer to give a third injection after 7 to 10 days For children the dose is proportionately reduced according to age.

This vaccine has been used extensively in the British Army since 1909 with very good results. The immunity lasts for about a year, and revaccination with a single dose of 1 ccm should be done every year. Intravenous inoculation of typhoid vaccine has been tried recently with very encouraging results. The immunity is said to last longer in these cases but owing to the danger of protein shock this method is not recommended.

Oral administration of vaccine has been extensively used in South Africa. The vaccine is given as a suspension in teaspoonful doses, or it can be made into a pill. Three doses each of 45,000 million organisms combined with ox bile in keratine-coated pills can be given. The results of oral administration however are inconclusive.

In the treatment of typhoid fever vaccines have been used with some success. An initial dose containing 100 millon organisms is given subcutaneously and subsequently three to four days apart, the dose is increased gradually; if sensitized vaccine is used this should be given daily for four days in doses of 500 to 1,000 million organisms subcutaneously or 100 to 200 million intravenously. From the available evidence it appears that typhoid vaccine may favourably influence the course of the disease, but it has been argued that the same results can be obtained by giving any other bacterial vaccine, and that the action obtained is most probably non-specific in character.

Bacillary dysentery. Two types of organism are mainly concerned in this infection, *Bact. shigae* and *Bact. flexneri. Bact. shigae* is a very toxic organism producing an exotoxin whereas *Bact. flexneri* is relatively non-toxic. On account of the toxicity of the Shiga type various attempts have been made to reduce the toxicity of these strains for the preparation of vaccine.

Recently, formolised vaccine (anacultures) have been used with success but it has not as yet been tried extensively enough to enable one to assess its real value in prophylaxis. Vaccination against dysentery with oral administration of Besredka's bilivaccine is recorded to have given good results but they are not so uniform as in the case of subcutaneous injection.

In the case of *Bact. flexneri,* the preparation of a vaccine is much less difficult as it is nontoxic. Except that the vaccine should contain all the representatives of various antigenic groups there is no other precaution necessary in the preparation of the vaccine. The usual prophylactic dose is 250, 500 and 500 millions at weekly intervals.

Treatment of bacillary dysentery by means of vaccines is used especially in chronic cases. Opinions as to the efficacy of vaccine in such condition are very divergent but Nolf (1919) and Acton and Knowles (1924) report favourable results with autogenous and stock vaccines.

In the beginning vaccines may be given subcutaneously or intracutaneously starting with small doses such as 10,000 to 1,000,000 organisms and increasing to as high as 5 to 10 million organisms. Some clinicians have reported striking results in chronic cases with intravenous doses every fourth or fifth day and gradually increased from 5 to 60 million organisms. It has also been tried in combination with 150 to 200 ccm of water per rectum by the drop method. Polyvalent vaccines have also been tried by the mouth. Doses recommended are 20 to 30 drops on the first day, 50 drops on the second day and 60 to 70 drops on the third day. Vaccines in the form of tablets have been prepared for use during epidemics. Each tablet contains 100,000 organisms and 5 or 6 tablets may be given daily dissolved in water or sodium chloride solution. The results of vaccine treatment have varied greatly in the hands of different observers.

Cholera vaccine. It is only used for prophylactic purposes. The vaccine consists of 24 hours culture of the cholera vibrio suspended in normal saline, killed by heat at 56°C. for half an hour and 0.5 per cent. phenol added as a preservative. Vaccines prepared in India contain 8,000 million organisms per ccm and the usual prophylactic doses are 0.5 ccm and 1 ccm at an interval of a week. A third dose of 1 ccm may be given but is thought to be unnecessary. Sometimes cholera vaccine is mixed with T. A. B. vaccine to produce immunity against all simultaneously. In emergency when two doses cannot be given, a single dose of 8,000 millions will give rise to sufficient protection.

In the prophylaxis of cholera, extensive observations were made in India by Russell. He found that in the comparative trial of cholera bilivaccine (oral) and the ordinary cholera vaccine (subcutaneous), both conferred a high degree of immunity. Russell expressed the opinion that the subcutaneous method was superior, and was able to show that five days after a single subcutaneous dose of cholera vaccine the immunity was about as high as that present three days after a full course of the oral vaccine.

Plague vaccine. Statistical evidence from different countries shows that vaccination against plague reduces the incidence and case mortality markedly.

"Formolised anti-Plague Vaccine (Plague Vaccine: Haffkine's Plague Vaccine).— Formolised Anti-plague vaccine is an uncontaminated culture of micro-organisms, *Pasteurella pestis,* grown in liquid media, *e.g.,* acid hydrolysate of casein and killed by the addition of formaldehyde. It should have a minimum mouse protection dose of 0.004 mils. or less.

"It may be prepared in the following way. A fresh sub-culture of *Pasteurella pestis* is selected which has been examined to ensure its identity and purity and which has been tested for virulence and antigenic efficiency by animal experiments. The culture medium used is an acid hydrolysate of casein having a pH of 7.2 and a nitrogen content of 250 to 270 milligrammes per 100 millilitres. A forty-eight hour culture of the selected strain of *P. pestis* is prepared in this medium, in a Pasteur balloon flask, and this is used to inoculate three litre Haffkine flasks each of which contains one litre of the acid hydrolysate medium. The Haffkine's flasks are incubated at 28° for 15 days. The cultures are then killed by the addition of solution of Formaldehyde, in a concentration of 0.1 per cent, which is allowed to remain for 7 days at 37°. Tests for purity and sterility are carried out at appropriate stages during the process of manufacture. The killed cultures are preserved by the addition of phenyl-mercuric-nitrate in the proportion of 1.5 milligrammes per 100 millilitres of the vaccine, or by some other suitable preservative. The finished vaccine is distributed, under aseptic conditions, into previously sterilised containers, which are sealed so as to exclude bacteria."

The vaccine is a brownish turbid liquid, with or without flakes or clumps. It complies with the tests for sterility, and tests for freedom from abnormal toxicity. Its potency is determined by the biological assay of plague vaccine. Anti-plague Vaccine should be stored at a temperature of 0.0° to 4.0°, and should not be used later than three years after preparation."

Lobar pneumonia.—A considerable amount of work has been done in recent years on the value of vaccines in the prophylaxis and treatment of lobar pneumonia and workers are unanimous that administration of pneumococcal vaccine confers a high degree of immunity in susceptible animals. The immunity is type specific so that in the preparation of a prophylactic vaccine all the common types have to be included. According to the recent work of Griffith on the variability of the types of pneumococcus there is a possibility of change in types after repeated mass inoculation so that the vaccine has to be changed accordingly.

Prophylactic vaccination for pneumonia has been extensively tried on the Rand mines in Africa with inconclusive results. The trials in the army have also not produced convincing evidence in favour of prophylactic vaccination.

As regards the treatment of lobar pneumonia with vaccine it has been seen that if administered early it influences the course of the disease so that combined with serum treatment it may be of great help. Minor pneumococcal infections such as the common cold with bronchitis, chronic sinusitis and other nasal inflammations if unattended with any other pathological condition, *e.g.*, deviated nasal septum, polypus, etc., are benefited by the administration of a pneumococcal vaccine. Where possible, autogenous vaccine should be employed but stock vaccine is also of benefit. In the conditions the initial dose is 10 million organisms increased every 3 or 4 days, up to 100 millions; after this it is increased by 100 millions at weekly intervals. For prevention of exacerbations, monthly injections of 100 millions should be continued during the winter.

Whooping cough. Results had been very variable with the use of this vaccine in the past when the importance of freshly isolated cultures was not recognised, but with the use of fresh cultures the results have been very encouraging. The vaccine must be prepared from an absolutely 'smooth' virulent strain, preferably a freshly isolated one. The vaccine is usually prepared to contain 10,000 million bacilli per ccm. Four injections are given at an interval of 7 to 10 days. The initial dose is 1 ccm, the second and the third doses are 1.5 ccm each and the fourth dose is 3 ccm.

For treatment, mixed vaccines are better than vaccines consisting of only *H. pertussis.* A combination of 500 millions of *H. pertussis* with 250 millions of *H. influenzae* and 20-100 millions of pneumococci per ccm is very suitable. This is given every day or every 2 days to a child of five or six years in doses of from 0.2 ccm to 1 ccm. Cockshut claims to have got extremely good results by the use of doses, 4 to 10 times of those recommended.

Influenza.

Influenza. For active immunisation in influenza Andrews has used formolised mouse adapted virus in England and Francis and Magett have used living culture virus in America. Human beings inoculated subcutaneously with both these preparations show a marked rise in antibody content. So far there have been limited trials in the field but the results in America appear to be hopeful.

Acute rheumatic fever. Vaccine treatment of this condition with hæmolytic streptococci has given favourable results especially in checking the recurrences which are so common in this condition. Weekly doses are given commencing with 200,000 cocci followed by 500,000 and then continued as follows:—1, 2½, 5, 10, 15, 20 millions followed by a gradual increase by 10 millions till 100 millions have been given. The vaccine was administered intravenously with safety.

Chronic rheumatic conditions. These include various forms of non-specific arthritis, fibrositis, perineuritis, etc. Various attempts have been made to treat these cases with vaccines prepared from organisms isolated either from the site of the lesion or from a septic focus in the teeth, tonsils, nasal sinuses, genito-urinary or intestinal tracts. Although it is very difficult to correlate the finding of organisms from such septic foci and the occurrence of rheumatic conditions yet it has been seen that vaccines prepared from them are able to alleviate or cure the condition in a large number of cases. Vaccine treatment should be auxiliary to any radical measures necessary for the removal of an infected focus. Autogenous vaccines are better than stock vaccines in these cases but the vaccine should be prepared by an expert. A stock vaccine for such conditions should contain a large number of strains from the mouth, tonsils, intestine and genito-urinary tract isolated from rheumatic cases.

The Arthritis Committee of the British Medical Association (1933) recommends an initial dose of 20,000 to 50,000 organisms when a septic focus is suspected or half a million when no septic focus exists. The doses are gradually increased every five or six days and it is rarely that a case will require a dose of more than 10 millions.

Acne vulgaris. This condition is due to a mixed infection with the acne bacillus and the staphylococcus. Vaccines prepared from both these organisms have been found to be of benefit in such conditions. The treatment should be continued for a long time to get any good result. Autogenous or stock vaccines may be used. The stock vaccine should contain both types of organism.

For therapeutic purposes mixed staphylococcus and acne vaccines are used. The dose of staphylococci is from 200 millions gradually worked up to 2,000 millions. Acne bacillus is best given in an initial dose of 5 millions followed at weekly intervals by doses of 7½ and 10 millions. If there is no improvement with these, the dose should be increased to 100 millions, and should be followed by increases of 100 or 200 millions up to 2,000 millions. As the acne bacillus is but slightly toxic and very seldom gives rise to general reactions these large doses can be given with safety. Sometimes it is mixed with intestinal streptococci especially in cases where there is a preponderance of streptococci in the intestine. The dose used is similar to that of the acne bacillus. This treatment should, of course, be combined with suitable medicinal, dietetic or physical treatment and regular evacuation of the contents of acne pustules and removal of comedones.

Furunculosis. Staphylococcus vaccine was the first vaccine used for therapeutic purposes by Wright. Usually staphylococci isolated from boils are antigenically similar so that there is very little difference between the use of a stock vaccine and autogenous vaccine. Vaccine in the treatment of furunculosis serves two purposes. Not only does it cure the condition but it prevents the recurrence of this condition anywhere else in the body.

Large doses are recommended for indolent boils to produce a focal reaction which is beneficial in such cases. The usual dose in a severe case is 100 to 2,000 millions of staphylococcus. In less severe cases 200 or 300 millions may be the initial dose, increased at intervals of 5 to 7 days up to 1,000 or 2,000 millions. Usually six to eight doses are sufficient to cure even very chronic cases. But, failing with this vaccine, streptococci isolated from fæces or boils may be combined with this vaccine.

Gratia (1930) has used a vaccine in which staphylococci were dissolved by certain streptothrices and moulds. This he calls *mycolysates* which are very beneficial in chronic cases. Bacteriophage lysed vaccines are also in use but the results so far are indifferent. As a result of the recent isolation of a powerful exotoxin from staphylococci, it is being used in modified form (toxoid) for the treatment of boils. This toxoid is prepared by incubating the toxin with 0.3 per cent. formalin for 2 days and injecting subcutaneously commencing with a dose of 0.05 ccm and increasing the dose every five to seven days by 0.05 ccm. Although still in the experimental stage, the results so far obtained are very encouraging.

Coliform infections. Bact. coli vaccines are used both for prophylactic and curative purposes. For prophylaxis they are used in patients suffering from coliform infections of the urinary tract preparatory to a major operation of the genito-urinary tract. Cases for such treatment should be carefully chosen as there may be acute symptoms of coli fever following an initial dose in cases where there is an obstruction to the outflow of urine. The doses recommended are 100, 500 and 1,000 millions at weekly intervals.

For therapeutic purposes these vaccines are used for cases of bacilluria and pyuria which are resistant to ordinary treatment and in which no exciting cause can be found.

The initial dose in children over 5 years should be 20 millions, increased at weekly intervals to 500 million organisms. In adults the initial dose is 25 millions, increased gradually at weekly intervals to 2,000 millions if there is no focal or general reaction. Acute infections due to late fermenting coliform bacilli are also treated with such vaccines. The initial dose of 25 millions should be given about 8 days after the temperature has returned to normal, the dose being increased gradually to 500 or 600 millions at weekly intervals.

Ulcerative colitis. Recently, a special type of diplococcus has been isolated from ulcers in cases of ulcerative colitis and vaccines prepared from such organisms either alone or combined with an antibacterial serum have been found to be of great benefit (Bargen 1924). The treatment however should be combined with strict attention to diet and removal of septic foci. The vaccine is prepared by culturing the cocci in broth for 48 hours, adding 0.4 per cent tricresol to the culture to sterilise it and the concentration adjusted to 2,000 millions per ccm.

An initial dose of 200 millions is given and if it is not followed by any reaction the dose is increased every third day up to the maximum dose of 2,000 millions per ccm. Sterile filtrates of cultures of the organism have also been used, the maximum dose being 1.15 ccm of such filtrate.

Gonococcal infections. The use of such a vaccine has been of great value in cases of complications resulting from an infection with gonococci, but used with caution it can be helpful in all stages of gonorrhœa. In the early stages it reduces the duration of primary infection and diminishes the number and severity of the complications. It is used in complications such as arthritis, epididymitis, orchitis, etc. Gonococci are composed of many antigenically varying members so that any vaccine to be of use should either be autogenous or should contain a large number of these strains.

Various different vaccines have been used for the treatment of such conditions but most commonly used is the simple vaccine prepared from freshly isolated cultures grown on serum agar. Mixed vaccines have been used in some cases consisting of staphylococcus, streptococcus or diphtheroid bacillus along with polyvalent gonococcal vaccine.

Only small doses are tolerated, and it is best to avoid any reaction. The dose also depends on the severity of the illness. In acute complicated cases it is advisable to begin with 500,000 organisms and gradually work up to 10 millions. In chronic cases the initial dose should be small but amount administered may rapidly be increased up to 500 millions or more so long as there is no reaction.

Asthma. True asthma is generally a result of hypersensitiveness to some foreign protein. There are, however, many cases in which this is associated with bacterial infection and it is in these cases that vaccine therapy has been found to be of considerable value by many workers. Either autogenous or stock vaccine can be used. For autogenous vaccine organisms such as *H. influenzae,* pneumococcus, streptococcus or *Bact. friedlanderi* are obtained from sputum culture. In a certain percentage of cases refractory to such vaccines, organisms isolated from the intestine, particularly enterococci, are incorporated in the vaccines with great benefit. In determining the sensitiveness to the various organisms isolated either from the sputum or intestine, dermal reactions are of great help.

The initial dose should always be small in order to prevent the precipitation of attack. The initial dose of streptococci, 0.1 to 0.5 million are used, increased by 0.1 million up to 1 million and further continued, where necessary, to as large a dose as can be tolerated by the patient.

If there is reaction with any dose, the next one is reduced and further increase in dosage should be made with great caution. Freeman advocates 0.5 ccm of adrenalin mixed with the vaccine to avoid reactions.

Smallpox. The vaccine used for prophylaxis is a living attenuated virus and of all prophylactic vaccinations this is perhaps the most useful. Vaccination has very appreciably reduced the incidence and the mortality rate of smallpox.

The vaccine is available in this country only in government laboratories and municipal vaccine depots. It consists of a glycerine emulsion of scrapings of pustules from calves inoculated with the smallpox virus.

The technique of vaccination. The skin of the forearm or upper arm is cleaned with soap and hot water and dried with a clean towel (no antiseptic is used). Two drops of the vaccine lymph are placed on the site of inoculaton and the part is gently scratched with a lancet over an area of half an inch by one-third of an inch, care being taken not to draw any blood. A blunt body like a small saw has lately replaced the lancet in many places. The excess of the lymph is allowed to dry on the skin.

Rabies. This vaccine has been found to be very successful for prophylaxis. The principle of vaccination in this case is the immunization of the affected person during the period of incubation which is fairly long.

Carbolised Anti-Rabic Vaccine (Carbolised Rabies Vaccine: Pasteur Treatment: Semple's Vaccine.)—Carbolised Anti-rabic vaccine is an uncontaminated suspension of brain substance containing fixed virus of rabies, inactivated by the addition of phenol.

It may be prepared in the following way. A suitable strain of rabies fixed virus is maintained by passage in rabbits. Healthy animals (preferably, sheep) are inoculated sub-durally with a suspension of the brain substance of a rabbit in which the fixed virus has been passaged. When the animal has died, or has been killed when moribund, after showing characteristic symptoms of infection with rabies fixed virus, the brain is removed under aseptic conditions, is tested for absence of bacteria, and is emulsified in physiological

solution of sodium chloride so as to form an 8 per cent suspension of brain substance. Phenol to make a concentration of 1 per cent is added and the concentrated suspension is incubated at 37° for 24 hours to kill the virus. The concentrated suspension is then diluted with physiological solution of sodium chloride to make, a final concentration of 5 per cent brain substance, and, the phenol content is adjusted to 0.5 per cent. The finished vaccine is distributed, under aseptic conditions, into previously sterilised containers, which are sealed so as to exclude bacteria.

It is a white or whitish. more or less turbid liquid having a slight odour of phenol. It complies with the tests for sterility and tests for freedom from abnormal toxicity. Carbolised Anti-Rabic Vaccine should be stored at a temperature between 2° to 10°, preferably at the lower limit. It should not be issued for use until after the lapse of at least 10 days from the date of addition of phenol. A test for presence of phenol must be made before issue. (Indian Pharmacopoea List, 1947).

Diphtheria.—In the past no method was known of immunizing children against diphtheria but with the increase in our knowledge on immunity various methods have been adopted for immunizing children against this disease. The formol toxoid and the alum precipitated toxoid are now used.

The recommendations of the Ministry of Health for immunization should be followed. They recommend that immunization should be preceded by a Schick test to exclude children who are already immune. Positive Schick reactors who have simultaneously been tested for hypersensitiveness to toxoid and found to be negative, should be immunized by subcutaneous injection of formol toxoid in doses of 1 ccm on three occasions with fortnightly intervals. Another Schick test should be done some months after immunization to ascertain the development of immunity. The most suitable age for such immunization is between one and three years as very few children are hypersensitive to toxoid at this age and it is given at a period when they are most liable to infection. Children below one year need not be immunized.

Recently 'one shot' method of immunisation and immunisation through instillation of the toxoid through the nose have been tried.

4. Serum Therapy

The practice of serum therapy began with the remarkable discovery of von Behring and Kitasato (1890) who showed that the sera of animals that had received injections of tetanus and diphtheria toxins had the property of neutralising these toxins and could prevent their poisonous effects. Following upon their discovery, Pfeiffer (1894) demonstrated that cholera vibrios, when introduced into the peritoneal cavity of immunized guinea-pigs, were killed and lysed. These observations lent support to the view that the blood sera of immunized animals contained protective substances which on being transferred to infected animals would help the latter to overcome the infection. Later on, attempts were made to prepare antisera against various bacterial infections and they were tried in the cure and prophylaxis of the corresponding bacterial diseases.

Although it was hoped in the begining that antisera of high therapeutic value could be prepared against various bacterial infections by immunizing animals, the experience of the past four decades has proved to the contrary. The reasons are not far to seek. There are various virus diseases, *viz.*, chicken pox, measles, etc., where the pathogenic organisms have not been culturally isolated in suitable antigenic forms, while there are others, *viz.*, typhoid, cholera, etc., in which though pathogenic organisms can be isolated, they fail to produce antisera of any therapeutic value.

A careful study of the available data reveals that the value of antisera varies with the type of the serum (whether antitoxic or antibacterial) and the time of administration in the course of the disease. For prophylaxis the usefulness of antisera seems to be limited. The protection they afford is only for a short period (4 to 6 weeks) owing to the rapid elimination and disappearance

of antibodies from the blood. As a therapeutic agent the use of antitoxins is extremely helpful, especially in diseases like diphtheria where the toxin circulating in the blood is directly neutralised by the antitoxin, provided it is given early and in adequate dosage.

On the other hand, antibacterial sera are not of as much prophylactic or therapeutic value as the antitoxic. This is because such sera act partly by the presence of specific bacteriolysins, opsonins etc., and partly by the mobilisation of the nonspecific immunity mechanism. Hence a higher concentration of antibodies in the serum does not necessarily mean greater efficiency. Still the administration of potent antibacterial sera in the early stages of severe bacterial infections, such as, Felton's antipneumococcus serum in type I pneumonia, polyvalent antimeningococcus serum in cerebro-spinal meningitis, in most cases cuts short the course of the disease and prevents unfavourable complications. It may, hence, be concluded that although the sphere of usefulness of antibacterial sera is limited, they are of considerable therapeutic value in certain selected cases.

Types and preparation of antisera. The antisera in common use may be divided into three types:

(1) Antitoxic sera, (2) antibacterial sera, (3) antiviral sera.

The methods of preparation of these are different and may be described briefly as follows:—

Antitoxic sera. These are prepared by immunizing horses by repeated inoculations of formolised toxins (0.2 to 0.4 per cent of formalin added to the crude toxic filtrate and incubated at 37°C for from 4 to 6 weeks). The serum is obtained from such animals and standardised in terms of antitoxic units as prescribed by the official control authorities such as the Permanent Commission of the Health Organisation of the League of Nations. Official control also exists in most of the countries to regulate the standard of purity and potency of therapeutic sera. The important and frequently used antitoxic sera are tetanus, diphtheria, gas gangrene, antivenin, etc.

Antibacterial sera. These are prepared by immunizing horses or other suitable animals by repeated inoculations of organisms. The antigen in this case consists of a suspension in physiological saline of the bacterium either living or killed, which contains both bacterial protein and endotoxin. The serum of the immunised animals contains antibodies against both. According to our recent knowledge on the antigenic diversity in certain types of organism and the extreme specificity in the action of the antisera prepared from such antigen, great care is necessary in the proper choice of antigen in the preparation of these antisera. In the preparation of a polyvalent antiserum, the different type specific sera are prepared separately and then are mixed together. The titration of such sera is done by estimating the different antibodies, *e.g.*, agglutinins, complement fixing antibodies, etc. or by animal experiments. Examples of sera mainly antibacterial are the antistreptococcal, anti-dysenteric, anti-pneumococcal, anti-meningococcal, anti-plague, etc.

Antiviral Sera. There is ample evidence to show that immune bodies capable of neutralising the virus appear in the blood during the course of virus infections such as measles, poliomyelitis, etc. These immune bodies can exert a prophylactic and some curative action. Convalescent sera have, therefore, been used in the prophylaxis and treatment of such diseases with favourable results specially in prophylaxis.

Patients free from tuberculosis, syphilis, or other infectious diseases are selected for this purpose and 500 ccm of blood from an adult or 100 ccm of blood from a child are obtained under sterile conditions, the serum separated, filtered and preserved by addition of 0.5 per cent carbolic acid or 0.39 per cent of tricresol. If kept in an ice chest, these sera retain potency for months. In measles in addition to serum from convalescent patients, serum from normal adults who have previously suffered from the disease has been used with considerable success. In view of the difficulty of getting large amounts of convalescent measles serum the observation is of great value. Normal adult serum has been tried for prophylaxis of measles but it is too early to evaluate the results. For prophylaxis purposes the convalescent or the normal adult serum is given intramuscularly within 5 days of exposure to infection and it is practically of no value if not given till 9 days after exposure. The dose of the convalescent serum in ccm is calculated by multiplying the age with 2, of the normal adult serum by multiplying the age with 4. For treatment the serum is administered by intravenous route and must be given within 6 days of onset. For prophylaxis of poliomyelitis 10 ccm of convalescent serum is injected subcutaneously or intramuscularly into young children who have been exposed to infection. The injection should be repeated after a month if the epidemic persists.

Convalescent smallpox serum has been used with promising results in cases of encephalitis following vaccination. It has also proved to be possible to immunize animals against virus infections and thereby to obtain a serum capable of influencing the course of the disease against which the animal has been immunized.

The concentration of sera. It has been found that the bulk of the antitoxin is associated with the pseudoglobulin fraction of the protein, whereas protective substances in antibacterial and antiviral sera are mainly associated with the euglobulin fraction. The different proteins of the serum, the euglobulin, the pseudoglobulin and the albumin, have different degrees of solubility in neutral salts. By adding appropriate concentrations of ammonium or sodium sulphate to the serum or plasma, different serum protein fractions can be separated for the purpose of concentration and purification of the sera. The advantage of such concentration and purification is that considerably larger quantities can be administered without the risk of foreign protein shock.

Storage and deterioration of sera. Sera must be kept in the dark and in the cold. The potency of most sera is maintained for a period varying from a year to two years after which there is a decline in the number of units of antitoxin in it, and if due allowance is made for this deterioration, sera may be used which have kept for periods longer than the time limit on the labels.

(1) Modes of administration

The choice of the route by which an antiserum should be administered is a very important consideration because upon it depends the rapidity of absorption of antitoxin or other antibodies. For prophylaxis where rapid absorption is not a necessity, antisera are usually administered subcutaneously. But the proper choice of route in the therapeutic use of an antiserum is of the greatest importance. The various routes most commonly chosen are subcutaneous, intramuscular, intravenous, intrathecal,and other less commonly used routes are intraperitoneal, intracisternal, intraventricular, oral and rectal. The best route to

be adopted in a particular case depends on the nature of the illness and the type of the antiserum, the severity of the illness, the stage of the disease at which antiserum is being administered, the age of the patient, etc.

Subcutaneous. This is the route commonly used although it is neither the best nor the most effective; for prophylaxis it is used very commonly. For therapeutic purposes, particularly in severe toxæmic cases, where rapid neutralisation of toxins is desirable, it is useless, because the antibodies inoculated are absorbed too slowly to attain quickly the necessary concentration in the blood. The belief that this route is safer than the intravenous route has not also been substantiated by experimental evidence.

Intramuscular. This route is preferable to the subcutaneous and should be more widely practised. When sera are given by this route antibodies are rapidly absorbed and reach a high concentration in the blood in a comparatively short time.

Intravenous. This is the best route for administration of antitoxin where the aim is to get a direct neutralisation of the toxins circulating in the blood. In acute and severe cases where toxæmia is great and delay dangerous, the initial doses of serum should be given intravenously as a routine procedure followed by intramuscular or subcutaneous injections for the subsequent doses. Experience has shown that this route is not generally more risky than others if the serum is given diluted with normal saline and at body temperature.

Intrathecal. This route is preferable in diseases such as cerebrospinal fever, tetanus and poliomyelitis where it is desired to obtain the highest concentration of antibodies in the focus of infection. A lumbar puncture is performed under local anæsthesia and an amount of cerebrospinal fluid equal to or slightly more than the amount of serum to be injected removed and the serum allowed to run into the theca by gravity or by injection with a syringe. The quantity of serum given at a time is generally 20 to 30 ccm for an adult.

Intraperitoneal. Plateau (1923) used this route in severe cases of diphtheria in infants where it was difficult to obtain suitable veins for intravenous injection. It has also been recommended by other workers and is said to be safe and well tolerated by the patients, but it has not been used much on account of the apparent risk of trauma or sepsis.

Intracisternal. This route of administration of sera has been employed to a considerable extent especially in America in the treatment of cerebrospinal fever (Goldman and Borwer, 1931). It appears to be free from danger but should not be undertaken without previous practice on the cadaver. The route is particularly suitable in cases with spinal subarachnoid block.

Intraventricular. This method has not been much used and it is doubtful whether the results are encouraging. Neal prefers this route to the cisternal route in cases with spinal block. In young infants ventricular puncture is best performed through the anterior fontanelle and the method is particularly useful in infants with a patent anterior fontanelle.

Oral. Although it has been shown that certain antisera are capable of exerting their specific effects when given by the mouth, the oral route is unquestionably the least effective. If this route is chosen at all, as for example in young infants, the serum should be diluted and given on an empty stomach in repeated doses.

Rectal. This route, although equally ineffective, is sometimes chosen to avoid serum reactions and discomfort following repeated inoculations. The rectum is cleared by an enema, the serum diluted with normal saline, made up to about 4 to 5 oz., warmed to body temperature and given high up with the help of a catheter.

(2) Reactions following administration of serum

Parenteral administration of a foreign protein is sometimes followed by certain reactions. These are usually of moderate severity and do not constitute any danger to life. The factors that bring about such unpleasant reactions are bound up in part with the serum, irrespective of the specific antibody it may contain and in part with the degree of sensitiveness to the foreign protein of the person receiving it. The following are the common reactions met with.

In most cases the injection of antiserum is followed by only slight reactions, such as local pain and tenderness accompanied by mild fever which passes off in 24 to 48 hours. In a small percentage of cases, however, more severe reactions may follow.

Protein shock. This is generally observed after intravenous injections of large quantities of serum and is ascribed to the effect of foreign protein introduced. The reaction sets in within 15 minutes to an hour and is characterised by chill, dyspnœa, cyanosis, rise of temperature, followed later by a fall of temperature to normal or subnormal, profuse perspiration and rapid pulse. The symptoms generally pass off without any treatment, but when they persist an injection of adrenalin 0.5 ccm or atropine 1/120 gr subcutaneously is all that is needed to restore the patient to normal.

Serum sickness. This complication is seen in some persons about 8 to 10 days and sometimes even earlier, after the injection of serum. It is characterised by urticarial rash, pruritus, pyrexia, swellings of joints and lymphatic glands, malaise, œdema and albuminuria. The incidence and severity of serum sickness increase with the age of the patient. The frequency of reaction is said to be less after intravenous than after intramuscular injections, and serum of some horses is particularly liable to produce these reactions. The reason for this is unknown. The reaction is rarely severe and needs only symptomatic treatment. Calcium lactate in 10 gr doses 3 or 4 times a day is beneficial; antipruritic lotions such as calamine lotion with 1.0 per cent carbolic acid relieve itching; aspirin 5 to 10 gr by the mouth reduces pain and pyrexia. When it is intended to give large doses, it is advisable, in order to avoid these unpleasant complications, to use concentrated sera in preference to ordinary sera. The incidence of this condition has been considerably reduced since the introduction of concentrated sera.

Anaphylaxis. This is one of the dreaded dangers that often dissuades the practitioner from using serum or giving it by the intravenous route. Anaphylaxis has undoubtedly been the cause of some fatalities, but it should be realised that the likelihood of its occurrence is remote, its incidence according to Park (1928) being only 1 in 20,000 and fatality 1 in 50,000. It can be avoided if a little care is taken and the case is studied before injection.

Some persons are extremely sensitive to the administration of a serum. These may be divided into two groups.

(*a*) Carriers of an hereditary allergic factor, *e.g.*, horse asthmatics who react almost immediately following the administration of a dose of serum.

(*b*) Persons sensitized by previous injection of a serum. The risk of death from allergic shock is very slight. But the administration of serum intravenously to horse asthmatics is more dangerous than to persons previously sensitized to serum. Although there is no risk of death the symptoms are sometimes alarming, characterised by a sudden onset, about a quarter of an hour after the introduction of the serum, with restlessness, anxious expression, pallor, perspiration, rapid feeble pulse, deep and laboured respiration followed by cyanosis, unconsciousness, muscular twitchings, rigors, convulsions and involuntary micturition and defæcation. For this reason it is necessary to test the sensitiveness of a patient by intracutaneous injections of 0.05 ccm of 1 in 10 diluted serum. If after 10 to 20 minutes the resulting wheal increases in size and shows a zone of erythema the reaction should be considered positive. The degree of sensitivity depends on the extent of the wheal and reactions with pseudopodial projections are indicative of an extreme degree of sensitiveness. In America the conjunctival test is preferred to the intracutaneous test. It is said to be a more sensitive test but unfortunately is associated with the risk of damaging the cornea in hypersensitive subjects.

Congenital sensitiveness to horse serum may be evident from history of the patient or may be revealed by a positive intracutaneous or ophthalmic tests. Such individuals should not be given horse serum under any circumstances or by any route. The recommendation of rapid desensitisation by half-hourly increasing dose of the serum is not fully justified. Immune serum from another species to which the patient is not allergic is the only way of securing the benefits of serum therapy for the congenitally allergic persons. It is also the most desirable form for the one who acquired horse serum allergy. Antiserum obtained from cattle may be suitable. Human immune serum has the great advantage of minimal liability to the production of allergic manifestations.

In other sensitive cases desensitization has to be carried on to allow introduction of serum in large quantities. This can be effected by (1) partition of the dose, and (2) slow administration of the serum.

For purposes of desensitisation 0.01 ccm of serum is given subcutaneously and this dose is doubled every thirty minutes till 1 ccm in all is given. After half an hour 0.1 ccm is given intravenously and then doubled every 20 minutes till the full dose is administered. If there are any allergic symptoms at any time, the previous dose should be repeated instead of increasing the dose further. Administration of the serum may be preceded by an injection of 1/150 to 1/100 gr atropine and adrenaline chloride solution (1 in 1,000) is kept ready for emergency purposes in case it is needed. The serum is given very slowly diluted and warmed to body temperature. When anaphylaxis comes on in spite of due care, symptomatic treatment should be given. Injections of adrenalin, pituitrin and atropine are useful. Inhalations of amyl nitrite or of oxygen may help.

Calcium may be given by slow intravenous injection of 20 ccm of 10 per cent solution of calcium gluconate. The rate of injection should not exceed 0.5 ccm per minute. It may be supplemented by 10 ccm of 10 per cent solution of calcium gluconate given intramuscularly and this injection may be repeated every twelve hours until the rash or other symptoms have subsided.

Febrile reactions after intravenous injections of serum. The reaction occurs about an hour after the intravenous dose and is characterised by rigor, increase in cyanosis in pneumonia cases, profuse sweating and a fall in temperature. It is not a serious complication. Care should be taken to use sterile distilled water for the preparation of normal saline necessary for diluting the serum.

Neurological complications of serum treatment. Although they are rarely met with, the presence of these complications has been recognised lately. They may be classified into four groups (Allen, 1931). (1) A radicular type, resembling an Erb-Duchenne paralysis of acute onset; (2) a neuritic type, in which single nerve trunks are affected; (3) a polyneuritic type in which the clinical condition resembles that of a toxic polyneuritis; (4) a cerebral type in which the prominent features are probably referable to intracranial œdema.

The condition has most often followed the use of tetanus antitoxin and the symptoms have appeared a few days after an attack of serum sickness. The patient complains of intermittent stabbing pains in one or more segmental areas or in the distribution of a peripheral nerve. Muscular weakness and wasting follow. This condition may persist for months but prognosis in such cases is generally good.

Dosage of antisera. The dose is dependent on so many factors that there cannot be any generalisation in the question of dosage. It depends on the type of serum, the severity of the disease, the period at which it is administered and the age of the patient. Each case is judged on its own merits; dosage has been discussed when dealing with the different antisera.

(3) Antitoxic and Antibacterial Sera

Diphtheria antitoxin. This is obtained by immunising horses against diphtheria toxin. Only the exotoxin is used for immunising purposes.. Such a serum is capable of directly neutralising the diphtheria toxin.

There cannot be any universally accepted scheme of dosage as in various diseases the interval elapsing between the onset of the disease and the time of administration of the serum have all to be considered.

The following scheme of dosage modified from 'Savilles' "System of Clinical Medicine" 1939, gives the units of antitoxin to be administered to cases of varying grades of severity.

Mild cases: 1 injection of 4,000 to 8,000 units.

Moderate cases: 2 injections each of 16,000 to 48,000 units.

Severe cases: 2, 3 or more injections each of 64,000 to 100,000 units.

The injections are best given intramuscularly into the vastus externus. Children tolerate the antitoxin well and should be given the same dose as adults.

It should be clearly borne in mind that the antibody can only neutralise the circulating toxin or possibly the toxin only loosely attached to susceptible tissues. Once the toxin is firmly attached to the tissues no dose of antitoxin, however massive, can exert any beneficial influence. We should, therefore, aim to introduce as much antitoxin as possible at the earliest possible moment for certain and rapid effect—one large dose given early is far more efficacious than divided doses. It is easy to give too little but it is very difficult to give too much.

The mode of administration is of great importance, as the object in antitoxin treatment is to attain an effective concentration in the blood with the least possible delay. The ideal method therefore is the intravenous for all cases and the next best is intramuscular in which the rate of absorption is 3 to 6 times greater than the subcutaneous. 'Platou (1923) suggested the intraperitoneal route in cases where it is difficult to find the veins particularly in infants and children. This route has not been chosen to any extent perhaps on account of the risk of trauma or sepsis. It is important to mention here the fact that the efficacy of antitoxin is greatest when given early in the attack and that it decreases as the day of administration is delayed.

Tetanus antitoxin. It is obtained by immunising horses against formol toxoid or alum precipitated toxoid. This anti-toxin is of great value in the prophylaxis of tetanus but its value in the treatment of the disease is very limited.

The prophylactic use of such a serum not only prevents the occurrence of the disease but also influences the character and progress of the disease in patients who subsequently develop it, increases the period of incubation and the incidence of local tetanus, thus lowering the rate of mortality.

For prophylaxis, the amount to be injected depends on the severity and the degree of contamination of the wound as well as the interval that has elapsed since its infliction. 1,000 to 3,000 international units (500 to 1,500 U.S.A. units) are given intramuscularly before any attempt to clean the wound by surgical methods is made. Ramon and Loeller (1927) recommended the following procedure: 10 ccm of antitoxin and 1 ccm of anatoxine (anatoxic formolised preparation) are administered at different places. Antitoxin is repeated ten days later and after another ten days 2 ccm of anatoxine is given. This method is said to afford protection in those patients who have incurred a risk of tetanus.

While the results of clinical trials of the therapeutic value of the anti-toxin after development of symptoms in man have not been conclusive the experiments of Sherrington (1917) on experimentally infected monkeys conclusively proved its therapeutic value. The dose of antitoxin used in Sherrington's experiments was very large 2,000 units per kilo body weight of monkey, this would correspond in man to 120,000 units given in a single dose. The route of administration of the serum was another important factor. Subcutaneous or intramuscular injection had but little effect, while intrathecal injections saved the lives of one half of the animals. The conclusions of Sherrington's work have been supported by clinical trials whereby it has been possible to cure tetanus cases by using very large doses of serum. Yodh (1932) obtained very encouraging results at the J. J. Hospital in Bombay. The total amount of serum administered to any patient varied from 40,000 to over 400,000 units. Intrathecal administration of the serum give better results than administration by any other route.

In the therapeutic treatment of tetanus, therefore, large repeated doses of the serum are required. The serum should be given by intramuscular, intravenous and intrathecal routes. There is evidence to show that the injection into the cisterna magna gives better results than the lumbar puncture route.

Antistreptococcus Serum.

Antistreptococcus Serum. There are two different types of sera, antitoxic and antibacterial, to be considered under this heading. The antitoxic serum is produced by immunising horses with erythrogenic toxin of *Str. pyogenes* and is known as the anti-scarlatinal serum. The ordinary antistreptococcal sera are prepared by immunising horses against killed cultures of streptococci.

Antiscarlatinal Serum.

Antiscarlatinal Serum. It is used in the treatment of scarlet fever. It is a specific antitoxic serum and on a priori ground one would expect that its administration will have an effect only on those manifestations of the disease which are toxic in origin and will have no effect in those complications such as otitis and adenitis which are caused by the massive spread of hæmolytic streptococci. There is evidence to show that the serum actually has a significant therapeutic effect on toxic manifestation of the disease as it decreases the death rate in severe cases and diminishes the toxaemic symptoms in milder cases. As regards its effect in reducing the frequency of complications most of the observers did not notice any such effect. But others found that the frequency of these complications was considerably reduced.

The antiscarlatinal serum, the chief effect of which is to neutralise the erythrogenic toxin, can not be expected to do any good in diseases such as puerperal fever, cellulitis, etc. Which are caused by the invasive spread of hæmolytic streptococcus.

Antistreptococcal serum. This serum is used in the treatment of puerperal fever, cellulitis, erysipelas, endocarditis, etc. The existence of a large number of antigenic types of the hæmolytic streptococcus makes it essential that to be of any use the serum should be type-specific, *i.e.,* it should contain antibodies corresponding to the surface antigen of the infecting type just as we use type-specific sera in the treatment of pneumococcal pneumonia. Since such type-specific antistreptococcal sera are not available there is no scientific basis for using the available antibacterial sera in the treatment of diseases caused by invasive spread of hæmolytic streptococci. The results of clinical trials have on the whole been rather disappointing.

Antidysentery serum. The chief bacterial species calling for serum treatment in the order of their importance are the following:—*Bact. shigae,* and *Bact. flexner.* Although there is some divergence of opinion on the efficacy of antidysentery serum, there is considerable evidence of a general nature to support its use. The best results are obtained with the Shiga serum, but the results are quite satisfactory with the use of Flexner serum also. In the preparation of antidysentery serum (Flexner) all the types of antigens such as V.W.X. (Y) and Z should be included in immunizing horses. The Flexner serum consists of antibacterial element only whereas the Shiga serum is antitoxic. Some people are of opinion that the efficacy of the serum increases if there is a sufficient amount of antibacterial component in addition to the antitoxin. Although Shiga serum can be standardised, there is no method available to standardise the Flexner serum.

Serum for therapeutic purposes should be given early (12 to 24 hours after the onset of symptoms) and in adequate dosage to get the best results. In cases where the nature of the dysentery has not been diagnosed it is best to use a polyvalent serum. In cases of ordinary severity a single injection may be followed by remarkable improvement, but in severer cases the dose has to be repeated in from 12 to 24 hours, and again in 48 hours. In Shiga infection 3,000 to 4,000 units should be given in mild cases and 5,000 to 10,000 in more severe cases. Serum in strength of 1,000 units per ccm is generally obtainable, but the more concentrated preparations contain about 5,000 units per ccm. The early stages of the disease are undoubtedly benefited by large doses of the serum and the death rate is low, but after the first week of the disease it is less effective and of no value in chronic cases. In patients suffering from severe toxæmia the best results were obtained by intravenous injection of 60 to 80 ccm of the Shiga serum followed by 150 to 300 ccm of normal saline, administered twice daily for the first two days and once daily for the next two days. In very toxic cases 5 per cent glucose in distilled water is substituted for the saline. In less severe cases the intramuscular route is preferable to the subcutaneous route, which is painful, and the dose should not be less than 40 ccm in an adult and as much as 100 ccm may be given.

High rectal injections of 10 to 30 ccm of polyvalent serum after cleansing the bowels with 1 5 per cent sodium bicarbonate and followed by starch and tincture of opium have also been tried. Intramuscular injections should be given at the same time. The varying results given by the serum are probably due to the difficulty in standardising and the varying qualities of sera on the market.

Antipneumococcus serum. This serum is prepared by immunising horses with whole cultures of pneumococci. The therapeutic effect of the antipneumococcal sera are strictly type specific. Extensive trials with antisera in the treatment of lobar pneumonia suggest that type I antiserum significantly reduces the mortality rate of type I pneumonia, that the results with type II antiserum in the treatment of type II pneumonia are less efficacious, and that the type III antisera are quite ineffective. Antisera against a few of the newer types (VII, VIII and XIV) have been prepared but they have so far been used on too small a scale to allow any conclusions to be drawn. It is essential therefore, that if an antiserum has to be used in a case of lobar pneumonia the type of the infecting pneumococcus should be determined as early as possible. To start with a polyvalent type I and type II antiserum should be given, when the type of the infecting pneumococcus is determined and if it happens to belong to type I or type II the polyvalent serum may be replaced by the monovalent serum if available or the polyvalent serum may be continued. If the infecting organisms is found to belong to the other types against which no therapeutic sera are available the injections should be discontinued.

Felton's serum is a concentrated serum containing antibodies against type I and type II pneumococcus.

The use of such a serum reduces the temperature, pulse rate, respiratory rate and the toxæmia. The inflanmatory change in the lungs is checked, but it has no influence either on the rate of resolution or on the complications.

The intravenous route is the method of choice for the administration of the serum. If there is any reaction it can be controlled by intramuscular or intravenous injection of 0.5 to 1 ccm of 1 in 1,000 adrenalin solution. The risk of allergic reaction following the first dose may be minimized by slowly giving 1 ccm of serum intravenously either pure or diluted with sterile normal saline. The rest of the dose is given after an interval of 20 to 30 minutes.

There is no uniform scheme of dosage, the dose depending on the severity of the case, and the time of administration in the course of the disease. Cecil advises an initial dose of 5 ccm followed in one or two hours by 15 to 20 ccm of the serum and a dose every 2 or 3 hours thereafter till 100,000 units have been given (usually 100 ccm). The doses are halved if the patient shows signs of improvement. An additional dose of 10 to 20 ccm may be given on the following day.

Antimeningococcus serum. It has been used extensively in the treatment of cerebrospinal fever for a long time. The earlier reports as to the efficacy of such a serum were somewhat exaggerated, but the present day opinion is that the serum, given early, controls the infective process and shortens the illness. The complications and sequelæ are much less; relapses are also less frequent and the disease does not continue so long. Although this is the usual experience with antimeningococcus serum, there are some cases where this form of treatment fails entirely. The reason for this is that the meningococcus consists of a heterogenous antigenic group and the serum to be effective must be prepared from a large number of representative strains from these different antigenic groups. Further it has been seen that strains isolated from sporadic cases are different from the epidemic strains and sera prepared from such strains are of little therapeutic value in epidemics. The best method therefore would be to collect a surplus stock of the serum during an epidemic period and to use it for the next epidemic till organisms are freshly isolated and fresh sera raised.

There is no known method of titrating this serum. The experiments of McKenzie and Martin have shown that substances bactericidal to the meningococcus are present in increasing amounts in the serum of acute and chronic cases of cerebrospinal fever and the serum of convalescents. Encouraging results have been reported on efficacy of such a serum in the treatment of cerebrospinal fever but technical difficulties have hindered its trial on a large scale.

Daily intrathecal injections of from 30 to 60 ccm for an adult and 10 to 20 ccm for infants have been found to give satisfactory results. At least 4 doses in all should be given, but there cannot be any hard and fast rule about the dosage since it depends on the severity of the illness and various other factors. Two points should be noted in its administration:—(*a*) It should be given as early as possible, (*b*) the amount administered should be less than the cerebrospinal fluid removed. The serum should be given warmed to the body temperature and by the gravity method. Two or three additional doses should be given after the disappearance of the cocci from the cerebrospinal fluid in order to safeguard against relapse.

As regards the route of administration, the intrathecal route is the best, but in predominantly bacteriæmic cases intramuscular injections of the serum may also be given in addition to the intrathecal injection. The intracisternal route is recommended in cases of subarachnoid block and is widely practised in the United States. It should not be undertaken by persons who have not had previous practice on cadavers.

Anti-anthrax serum. Anthrax is primarily a disease of animals and man is attacked only when engaged in an occupation which brings him in contact with such infected animals or their products. Of the three clinical forms of the disease the cutaneous type is the only one in which the administration of an antiserum is of some benefit. Although many different types of sera have been prepared and used from time to time, Sclavo's serum, which is perhaps the earliest preparation (1895), is the best.

Sclavo's serum is given in doses of 30 to 40 ccm subcutaneously followed by a similar dose after 24 hours if the local lesion or the general state of the patient does not improve. In severe cases 10 ccm should be given intravenously and repeated after 2 or 3 hours. In addition subcutaneous doses should be given as well. It should be noted that the best results are obtained by the administrations of the serum at the earliest possible moment.

Antityphoid serum. Although attempts have been made to use antityphoid serum in the prophylaxis and cure of typhoid fever, the results have been very contradictory and the value of such treatment is extremely doubtful. Gross (1930) prepared a necrotizing toxin from *Bact. typhosum* and he used this toxin for immunizing horses. The antitoxin so prepared is capable of producing both a prophylactic and curative action in mice. Besides the antitoxin it also contains agglutinins, complement fixing and bactericidal bodies. Reports on the clinical use of such a serum are not encouraging.

Antistaphylococcus serum. That the pathogenic staphylococci, particularly *Staphylococcus aureus,* may be associated with a toxin has been known for a long time, but the disaster at Bundaberg in Queensland in 1928, when 12 children died within 2 hours after being inoculated with a diphtheria prophylactic consisting of toxin-antitoxin mixture, which had become contaminated with staphylococci, led to renewed interest in the subject.

Parker (1924) showed that certain strains of Staphylo aureus when grown under suitable conditions produce a filterable toxin having a haemolytic, skin-necrosing, lethal and leucocidic properties. An anti-toxic serum capable of neutralising all these properties of the toxin can be prepared by immunising horses first with formolised and later with pure toxin. Puerperal infections caused by the staphylococcus have been treated with large daily doses (100 ccm) of this serum in Germany. It should be used as a precautionary measure in cases of furuncles of the lip, carbuncles, puerperal infections. The use of staphylococcal toxoid in the treatment of furunculosis has already been discussed in the section on vaccine therapy.

Antigasgangrene serum. The importance of this condition in the infection of wounds was recognised in the last War. Wounds grossly contaminated with soil containing anærobic and ærobic organisms are most likely to show signs of gas gangrene. Antiserum prepared against *Cl. welchii, Cl. œdematiens* and *Cl. novyi,* which are the commonest infective agents in such wounds, was extensively tried both for treatment and prophylaxis and has been found to be of great value particularly in prophylaxis. Gas gangrene is not so commonly met with in civil practice but in the likelihood of a wound being infected with anærobic bacilli, a polyvalent antiserum should be injected as a prophylactic measure. The serum given intravenously and injected into the deep tissues near the wound has been found to give satisfactory results and as much as 1,000 ccm may be required. It is most important to treat wound surgically in addition to the serum treatment.

This serum has also been used in acute abdominal conditions such as appendicitis and intestinal obstruction. Williams recommends administration of 80 ccm of the serum intramuscularly and an additional 40 ccm intravenously in very severe cases of intestinal obstruction. On subsequent days 40 to 80 ccm should be given intramuscularly until the distension has disappeared and the bowels are moving spontaneously and regularly. A prophylactic dose before operation is advisable. Its administration as an adjuvant to operative measures has proved to be of great benefit. It has also been used in the treatment and prophylaxis of puerperal sepsis due to infection with these pathogenic anærobic organisms. For such infections Wrigley recommends the use of 40 ccm of the concentrated specific serum for prophylactic purposes. Other conditions in which this serum has been found to be useful are perirectal abscess, gangrene of the lung and diabetic gangrene. The use of penicillin gives excellent results.

(4) Antiviral Sera

Measles. The cause of this disease is a filtrable virus which is present in the blood and nasopharyngeal secretions during the acute stage of the disease. It has not been possible to obtain an antiserum by immunizing animals so that human convalescent serum is needed

for prophylaxis. There are two types of protection:—(*a*) Full but temporary protection; (*b*) incomplete protection resulting in a mild attack of the disease. One attack of the disease confers a lasting immunity.

Full protection may be needed for children in a poor state of health, particularly those below three years, for children suffering from tuberculosis, rickets, diphtheria, whooping cough or scarlet fever if exposed to the risk of infection. The immunity lasts for 3-4 weeks and child is susceptible to infection after that period.

The effective dose for such a purpose depends on, (1) the age of the child; (2) the state of his health; and (3) the interval between the time of exposure to the infection and the time when the serum is given;this should not be more than five days. The average minimum dose is 5 ccm and the dose for children over three years of age is determined by multiplying the age of the child by two and the result gives the number of c.cm. of serum to be administered. The duration of the protection so conferred depends on the time of its administration. If it is given before exposure the immunity lasts from 2 to 4 weeks, but if given early in the incubation period, some degree of active immunity may be superimposed upon the passive immunity and if this happens the patient will be protected for a few months.

Convalescent serum, normal adult human serum and whole adult blood are three different agents used for such protection. It is not always easy to obtain convalescent serum so that normal adult human serum from persons who had an attack of measles in their childhood may be used with the same result, but it has to be used in large doses. The use of adult whole blood has the advantage that children can be injected with the blood from their parents when it will be unnecessary to perform a Wassermann test. The adequate dose of adult serum is 12 to 25 ccm.; of the whole blood 25 to 50 ccm are needed to confer full protection

Owing to difficulty in obtaining sufficient quantity of serum use is now being made of human placental extract. If the use of the placental extract proves successful it would be possible to immunise the children on a large scale.

Incomplete protection or attenuation as it is called is obtained by first exposing the child to infection for example by letting him run about with a case of measles for a day or so and then giving him an attenuating dose. The attenuating dose is the same as the dose for complete protection if given within 6-9 days after exposure or half that dose if given within 1—5 days after exposure. Unless contra-indicated by the weak health of the child it is much better to aim at attenuation rather than at complete protection because the mild attack of the disease will confer a lasting immunity. Attempted attenuation is, however, difficult because one has to be sure that there has been an intimate and close contact with a case.

Acute anterior poliomyelitis. Human sera from three sources are used:—(1) Convalescent serum, (2) serum from persons who had an attack previously even up to 10 or 20 years; (3) serum of normal adults with no history of having suffered from the disease. There is evidence to show that sera of persons who have never suffered from poliomyelitis contains immune bodies probably as a result of an acquired immunity due to sub infective doses of the virus in childhood.

From 10 to 20 ccm is given at the earliest possible moment intrathecally, and this is followed immediately by a dose of from 40 to 200 ccm. intravenously. The intra-thecal dose may be repeated on two or three successive days, but there is no necessity for repeating the intravenous dose. There is no danger in injecting human serum intravenously, as it is rarely followed by unpleasant sequelæ. It is no use giving the serum after paralysis has set in.

Prophylactic inoculation can be done either by human antiviral serum or by immune serum from horses. Flexner and Stewart (1928) suggest a subcutaneous dose of 10 ccm. for children and 20 ccm for adults repeated after 4 or 6 weeks if exposed to infection.

Chicken Pox. Although a comparatively harmless disease, prophylactic measures are justified due to the long incubation period necessitating a prolonged quarantine. Convalescent serum has been used for this purpose.

Mumps. Convalescent serum has been used both for prophlyaxis and alleviation of symptoms and complications of this disease. The results so far obtained are encouraging but not convincing.

Dengue. Convalescent serum has no protective action when given prophylactically or in the treatment of the disease.

Antivenin. This is an antitoxic serum against snake venom and considering the large number of deaths occurring in India from snake bite, it should be one of the most useful antisera. Unfortunately however there are so many practical difficulties in the preparation and use of this serum that it has not so far been very successful. For details see chapter on snake venom.

5. Bacteriophage Therapy

The discovery of bacteriophage is of comparatively recent date. Twort (1915) in a paper entitled "Investigation on the nature of ultra-microscopic viruses" first noted the existence of such an agent. d'Herelle, working at the Pasteur Institute, Paris, discovered a curious phenomenon in sterile filtrates of stools of patients suffering from the Shiga type of dysentery. The unique feature of this phenomenon, and one outside the realm of previous experience, was that the lysis produced by filtrates in cultures was transmissible in series *ad infinitum* without exhaustion of the lytic principle. This lytic principle was named *Bacteriophagium intestinale* or *Protobios bacteriophagus*. d'Herelle (1917) considered the bacteriophage to be a minute, ultra-microscopic, living organism, parasitic upon, and lytic towards bacteria and he has since done considerable amount of work in support of this theory.

Although bacteriophage, as we now know it, has been known for about thirty years, there are to be found indications of appreciations of its existence in the past. Most discoveries have their roots in the past. In certain parts of India the droppings of birds have been used for centuries past in the treatment of dysentery and other intestinal diseases; the use of cow-dung spread on the floors of kitchens as a purifying agent, was at one time, and still is, in many, if not all, parts of India, a common routine in the households. These practices were undoubtedly based on experience and clinical observations of many generations. The droppings of birds have been shown to contain very powerful bacteriophages against the organisms of dysentery, at times against the cholera vibrio, and even the typhoid group of organisms. The spreading of cow-dung to 'purify' the kitchen floors, etc., although now shrouded in the rituals of religion, is an efficient, though undoubtedly a very primitive form of disseminating bacteriophage. The drinking of river waters, the administration of certain waters to the sick would also appear to be a method of bacteriophage prophylaxis and bacteriophage therapy.

Hankin (1896) drew attention to the purifying effects of the river water. He noted the bactericidal properties of the waters of the Jumna. At Agra, for instance, be found that the river water contained 100,000 bacteria per ccm, whereas 10 miles further down there were only 100 per ccm. This observation remained unexplained till the discovery of bacteriophage. Other workers also observed many peculiarities in cultures of intestinal organisms. Gildemeister (1917) observed the nibbled appearance and defective colonies of dysentery and coli organisms without recognising their abnormality. This we now know as a phenomenon of bacteriophage.

(1) Nature of bacteriophage

Opinions differ regarding the nature of bacteriophage. A controversy as to whether it is a living organism or a dead product of a living organism is still raging. But the consensus of opinion tends to regard it as a living organism. If we consider the size of bacteriophage it will be appreciated how very difficult it is to settle the question of the nature of such an object. Bacteriophage is ultra-microscopic, *i.e.*, not visible by the most powerful mircoscope. Recent studies show that phage is composed of particles whose size is uniform for any phage, though varying from phage to phage. The smallest phage has a diameter of 8 to 12 , while that of the largest phage varies between 50 to 75.

According to d'Herelle bacteriophage is an autonomous, living, strictly parasitic organism belonging to the group of filterable viruses, which multiplies at the expense of, and causes the dissolution of bacteria. He is of opinion that there is but one bacteriophage with an infinite number of strains. To this parasite he has given the name of *Protobios bacteriophagus*. Other workers do not believe that bacteriophage is a living ultramicroscopic organism. Many consider that bacteriophage is a ferment, either living or dead. Kabesima (1920) considers it to be a secretion from the intestinal glands that acts as a catalysator on the proferment contained in all bacteria and causes their digestion. Bail (1921) formulated the hypothesis that bacteriophage is a disintegration product of bacteria, probably

derived from the chromosomes. Bordet and Ciuca (1921) consider that the phenomenon of bacteriophage is due to an inherited autolytic power on the part of bacteria. Hadley (1928) is of opinion that bacteriophage, as it occurs, is a definite stage in the life cycle of all bacteria or that it is an essential constituent accessory to the various stages. Otto and Munter (1924) state that it is a ferment-like action of the colloidal particles of bacterial cytoplasm.

Phenomenon of bacteriophagy: lysis and mutation of bacteria.

The phenomenon of bacterial lysis has been extensively studied in the case of cholera and plague. The stool of a patient recovering from cholera contains phage, and if a trace of such filtrated stool is introduced into a fairly turbid culture of cholera vibrios, this culture becomes perfectly clear after some time, all the vibrios being dissolved. Under the miscroscope this destruction of vibrios can be watched. It consists of swelling and spherulation of the organism, production of granules and finally disintegration of the bacteria. The clear liquid can again be inoculated into a fresh culture to obtain the same effect and the process can be repeated indefinitely. This illustrates the important characters of lysis of bacteria by bacteriophage, *i.e.*, its filterability, transmissibility and that it can be subcultured in series with living and growing bacteria.

The phage action can be observed in young bouillon cultures of the cholera vibrio as well as in other media. If an agar plate is thickly inoculated with culture of cholera vibrio so as to give a confluent growth and a few drops of a suitable dilution of the phage-containing filtrate is spread over this inoculated surface before the plate is inoculated there will appear a number of well separated clear, more or less circular areas within which there has been no growth. These areas are generally called plaques. These are the colonies of bacteriophage. They vary in size from that of a pin head up to 4 to 5 mm; small-sized phages give rise to large plaques and large-sized phages to small plaques.

If the plates with their areas of plaques are further incubated, it is found that, after a certain time, small colonies develop in these areas. A similar phenomenon in the form of return of turbidity is observed in bouillon cultures rendered clear by phage. The explanation is that after a certain time secondary colonies of bacterial cultures develop which consist of strains of bacteria resistant to the action of phage.

The adaptation of bacteriophage to produce intense lytic action on bacteria is important. It is more commonly adapted to one particular species of organism though its affinity for more than one species has been observed. Therapeutically active phage should, therefore, be highly lytic towards the specific organism. This adaptation of phage towards the specific organism can be brought about by growing it with specific bacterial species till the lytic power is developed. If, however, the phage is originally weak the lytic power can be augmented by repeated passage, with a substrate organism. This lytic power of phage is not unlimited, and the importance of the adapatation of bacteriophage towards bacteria can be realised from the fact that on it depends to a great extent the success or failure of the struggle that is going on between bacteria and the defensive mechanism of the host.

Bacteriophage is one of the most powerful agents in bringing about changes in the morphology, cultural and serological reaction of bacteria. These reactions are known as mutation and dissociation of bacteria and consist in alteration of motility and morphology, capsule formation, proteolytic power, agglutinability, virulence, formation of filter-passing forms, etc. The main results of such changes are development of resistance to lysis, greater viability, and increased susceptibility to phagocytosis. According to d'Herelle, these alterations are brought on by bacteriophage, and are evidence of a reaction on the part of bacteria towards a pathogenic organism which in this case is bacteriophage itself.

The modification of bacteria in this way may be so complete that secondary cultures from the original strain may be morphologically quite different. D'Herelle described extremely resistant and stable forms of cocci obtained by repeated transfers of young secondary cultures of dysentery, typhoid, paratyphoid, coli and cholera organisms. Hoder (1925) stated that there is definite possibility, of transition of *Bact. coli* to paratyphoid-like forms. Doorenbos (1932) claimed the EI Tor vibrio as a phage-modified form of cholera vibrio.

The conversion of non-hæmolytic organisms to hæmolytic forms, of agglutinating to non-agglutinating species by the action of bacteriophage, has been described by many workers. In the case of cholera, Pasricha and his co-workers (1931) state that there is some bacteriophage as well as serological relationship between the true cholera vibrio and the cholera-like forms. Many of these latter forms are considered to be only mutation forms of the true cholera vibrio and play great part in the incidence of cholera. Morrison (1931), on the other hand, concludes from his experiments that the alterations in the cholera vibrio are due probably to the occurrence of bacterial contamination.

Specificity and types of bacteriophage. Burnett (1933) stated that bacteriophages are independent micro-organisms and differ widely in size and activity. d'Herelle considers that there is only one species of phage but that it is capable of a high degree of adaptation to pathogenic organisms. There may be races of phage with a specific affinity for a certain strain or a species of bacteria to the exclusion of all other strains of the same species, which procedure has been regarded as a valuable aid to the diagnosis of bacterial species.

Several types of bacteriophage have been recognised especially with regard to cholera-phage. This is dependent upon the fact that with the development of a resistant series of bacteria on culture, a bacteriophage may be discovered which lyses these resistant strains. In this way, several types of cholera phage have been recognised. In India, Asheshov called his types A, B and C; Pasricha and his co-workers (1932) have added types D, E and F to these already known ones.

(2) Preparation of bacteriophage

The technique of bacteriophage study and the general principles of bacteriophage are so highly specialised that they involve special methods and what first appears to be a highly complicated technique. In reality it is perhaps one of the simplest techniques in the whole subject of bacteriology. It must be remembered that we are dealing with an organism which is so small in size that it passes filters which hold back all microscopically visible forms of life. This extreme minuteness of the bacteriophage corpuscle is of very definite advantage because it makes it possible for the bacteriophage to be separated from all visible forms of life by the simple process of filtration. Bacteriophage also possesses an additional property which is of extreme value in its preparation and study. It can only grow and multiply at the expence of living susceptible bacteria. Its food is the living bacteria and so far it has not been possible to devise any method for its propagation except on living material and those of its host.

Technique of filtration. This varies according to the nature of the fluid required to be filtered. It is generally done in the following way. Sterilised funnels with filter paper are filled with kieselguhr suspension. This leaves a very thin coating of kieselguhr on the filter paper, which retains most of the bacteria, giving an almost crystal clear solution. This clear filtrate is now filtered through porcelain candles under vacuum of 15 inches.

Isolation of bacteriophage. In order to isolate bacteriophage from stools, about 5 ccm of the stool, if liquid, or a small portion if solid, is mixed in a small flask containing broth (papain broth or any other liquid medium). If it is thought that the bacteriophage present is minimal, a few ccm of young peptone water culture of the organism, against which bacteriophage is sought, are added. The addition of fresh culture is made with the object of giving the bacteriophage present the opportunity to develop and thus to be more readily found.

Isolation of bacteriophage from water is done by following the same procedure. Large amounts of water may be necessary, and often preliminary filtration is required before the addition of 'enriching organisms.'

(3) Role of bacteriophage

The phenomenon of death, recovery and immunity from diseases has been explained on the basis of development or absence of bacteriophage. d'Herelle believes that such a phenomenon is not dependent upon immunity, as is generally understood, but on the occurrence of bacteriophage. In the case of cholera, cases which do not develop bacteriophage die; cases with feeble bacteriophage, which disappears, also die; cases with strong bacteriophage at the outset recover promptly; cases with weak bacteriophage, which increases in potency, recover with some delay and the ups and downs in the clinical progress of the case represent the behaviour of the bacteriophage. Bacteriophage by infecting bacteria, causes destruction and death of bacteria and is the direct agency of recovery of disease in man. By an indirect process it brings about a state of acquired immunity. Bacteriophage, by causing a solution of the bacteria, presents the bacteria in a condition to serve as ideal antigens. The lysates contain the lysed bodies of bacteria and induce in the body the mobilisation of the defensive forces.

If, however, the bacteriophage is not powerful enough or conditions are not suitable for the total destruction of the invading bacteria, then one of the two things may occur:—

(1) The bacteria may overcome the infection by bacteriophage, and develop an immunity against that particular bacteriophage and persist as saprophytes; in this condition they are entirely non-pathogenic. The question now arises whether these bacteria under any circumstances again become virulent and pathogenic. There is no direct evidence to either admit of this possibility or to define the conditions necessary for such changes to occur. It appears to be highly probable, however, that such changes do occur in nature and it is only rational to conceive that such changes should be the rule rather than the exception.

(2) The bacteria may be able to completely rid themselves of the infection by bacteriophage, and although they develop an immunity against that particular phage, they lead a life in harmony with bacteriophage. There is a symbiosis between the causative organism and bacteriophage and this results in attenuation of the virulence of the bacterium; such bacteria cause chronic disease. Acute diseases are due to young healthy disease-free or bacteriophage free bacteria; chronic diseased conditions are caused by the diseased unhealthy phage-infected bacteria which, inspite of their disability, yet retain sufficient invasive power to maintain a chronic diseased condition.

Such is the line of reasoning which has been advanced, but from the very nature and number of variable factors present, the proof of such conditions actually occurring is not very convincing. *Bact. typhosum* in its active pure stage causes typhoid fever provided certain conditions exist under which it can exert its full invasive power, but *Bact. typhosum* contaminated with bacteriophage—the organism of the 'carrier state'—may only cause a chornic infection such as cholecystitis. *V. choleræ,* when pure and healthy, kills a high percentage, the mortality rate is low or may cause very mild symptoms when infected with phage. *Past. pestis* kills rats when free, but when diseased it causes only a chronic form of plague. The theory of bacteriophage can even embrace complete consistent theory of acquired immunity, but these ideas should not be stretched too far in our present stage of knowledge.

(4) Bacteriophage in prophylaxis and treatment of disease

d'Herelle has shown a very definite relationship between the occurrence of bacteriophage in nature and the incidence of certain intestinal diseases, particularly cholera and dysentery. At the beginning of the cholera season very few samples of waters collected from the river Hooghly and from the tanks in Calcutta show the presence of choleraphage. During the height of the cholera season the incidence of bacteriophage in nature increases till about 40 to 50 per cent of the samples of water show the presence of powerful choleraphages. At the end of an epidemic, the whole bacteriological picture changes; instead of the cholera vibrio possessing typical characters which may be likened to a well-organised army, there are towards the end of an epidemic, heterogenous groups of organisms varying in their power, some difficult to recognise as vibrios, which are like the ragged disorganised members of a worn out army. All the factors that bring about this change are not known, but it is known that this enemy of man, the cholera vibrio, has been in conflict, amongst other forces, with bacteriophage which has played an important part in disorganising the invasion of the cholera vibrio. The course of a cholera epidemic, is a struggle between vibrio and phage for, dominance, and the ups and downs of all epidemics are the visible effects of that stupendous invisble battle that takes place in nature. The battle-ground is the intestine of man.

This concept of prophylaxis and immunity is so radically opposed to our ideas of epidemiology that at present it is difficult to appreciate its importance. It must be realised now that there exist in nature two very distinct forces—one, certain bacteria which are harmful to man and that source of these harmful bacteria in the majority of instances is man himself; the other, another living force, which because it is harmful to bacteria is of distinct value to man. This second

force is, as it were, provided by nature to maintain a state of equilibrium between man and his bacterial enemies. It is a police force provided by nature to guard mankind in times of stress. If conditions are favourable this force of bacteriophage multiplies so readily and becomes so powerful that it brings to an end the supremacy of the bacteria.

These theories raises very important points both in the prophylaxis and treatment of epidemic diseases. From this point of view the question arises whether the propagation of bacteriophage is advisable. To do this are we to allow the unhampered dissemination of natural bacteriophage which would necessitate the cessation of all public health activities, and to rely entirely on the results of the natural adjustment between the bacteria and the bacteriophage? These last factors may be so variable that it would not be justifiable to allow the cessation of the stringent sanitary measures. The bacteriophage as it exists in nature has had centuries of free play, unhampered by any sanitary measures, and we still have big epidemics of intestinal diseases. Secondly, the bacteriophage as they exist in nature may be supplemented by specially trained and highly virulent bacteriophages. In this way it may be possible to marshal together the natural enemies of bacteria, and harness nature for the benefit of mankind. This certainly must appeal to everyone as the most rational point of view. Experiments in the laboratory and trials with bacteriophage in the field, both as a prophylactic and therapeutic agent, have been undertaken by several workers, and although bacteriophage prophylaxis and therapy have made great progress, the subject is still under trial and no definite conclusions can be drawn from the experiments reported so far.

It can be definitely stated that the results of the trials so far are not against bacteriophage; there is evidence highly suggestive that bacteriophage is of value. Though great hopes were raised at first of the therapeutic value of bacteriophage, the results so far have not fulfilled these hopes. Great things are still expected from bacteriophage which on purely theoretical grounds possesses all the attributes of an ideal antiseptic. It has no action whatsoever on any living tissue, kills the invading organism rapidly and increases in amount as it does so. The conditions within the body may and do modify the effectiveness of its attack, but it is hard to believe that an active phage can be entirely without effect on the course of an infection by a sensitive organism.

Therapeutic uses. As early as 1921 bacillary dysentery was treated by administration of bacteriophage and since that time various results were obtained with this form of therapy.

According to d'Herelle, in the bacteriophage there is a natural therapeutic agent which is of value in the cure of disease as well as in prophylaxis. It has already been pointed out that an active specific phage possesses all the attributes of an antiseptic, and it kills the specific organism against which it is employed and increases in amount during this process. From this point of view it was considered a valuable therapeutic agent in bacillary dysentery, cholera and various other bacterial diseases. Unfortunately the success with bacteriophage treatment is not uniform and its usefulness has been disputed by many workers. It is said that the action of bacteriophage is brought about by purely chemical means and that the effects produced are nothing but a reaction due to the introduction of a foreign protein. Topley, Wilson and Lewis (1925) used bacteriophage in strictly controlled experiments with mouse typhoid infection. Their observations were that the presence of bacteriophage does not prevent the spread of infection,

check an epidemic when it once started, or appreciably reduce the mortality. They further found that there is no evidence of development of immediate immunity either from injection or ingestion of bacteriophage.

Recently however, Asheshov, Wilson and Topley (1937) using a phage active against vi strains of *Bact. typhosum* have shown in their experiments on mice that a phage may be active *in vivo* as well as *in vitro*. They are of opinion that under suitable condition, a phage that has a specific action on the surface antigen of a virulent bacterium is capable of exerting a significant protective action when it meets that bacterium in the tissues.

The majority of those who are however willing to accept d'Herelle's hypothesis are of opinion that bacteriophage is a living ultramicroscopic virus which is capable of being a parasite on bacteria and which is said to dissolve and destroy them through the agency of a ferment it secretes. In addition to its direct action on bacteria, bacteriophage may also exert an indirect action by increasing the phagocytic power of the leucocytes, the explanation being that the lysins secreted by bacteriophage are really of nature of opsonins. The cultures of bacteria lysed by bacteriophage have been considered to act as highly effective vaccines because of their capacity to produce antibodies possessing a far more protective power than the antibodies obtained from ordinary bacterial vaccine. The bacterial split products obtained under the action of bacteriophage are in a physical state highly suitable to induce a strong and durable immunity so that this immunity adds indirectly to the value of bacteriophage as a therapeutic agent.

According to d'Herelle many of the unsuccessful results of bacteriophage therapy are due to the inadequate technique in the preparation of phage. Only the potent phages are therepeutically effective.

The role of bacteriophage as a therapeutic agent is as yet complex. Satisfactory results can only be obtained by following certain procedures. It is to be understood that there is an essential difference in principle between the treatment of acute and chronic infectious diseases with bacteriophage. In cases of acute disease a powerful bacteriophage must be brought into contact with the pathogenic bacteria before they have had opportunity to produce sufficient lesions to cause death. Chronic cases possess a different aspect altogether from the point of view of bacteriophage treatment. In this condition a state of partial symbiosis exists between the infecting bacteria and the phage and in order to bring about a full therapeutic effect a race of bacteriophage has to be employed which is virulent for the bacteria.

The mode of administration and dosage of bacteriophage requires some consideration. Bacteriophage, to be of any value, should be administered as early in the course of the diseases as possible. Bacteriophage must come into direct contact with the invading organism and hence in intestinal disorders it should be given by the mouth. In cases of infection of the urogenital tract bacteriophage has to be introduced directly into the bladder, localised infections of subcutaneous or deep tissues required the direct application of bacteriophage in the infected focus. With regard to dosage and frequency of treatment it may be stated that the amount of bacteriophage to be given depends upon the virulence of the bacteriophage employed. This is due to the fact that bacteriophage in the presence of susceptible organisms perpetuates itself and the amount administered does not determine the amount that will ultimately develop. In case of acute infections a few administrations may be sufficient while in chronic types of infection bacteriophage therapy may have to be continued over a long period.

Bacillary dysentery. The treatment of bacillary dysentery with bacteriophage is now widely recognised. Fletcher tried bacteriophage in 22 cases of Flexner infection, but the treatment was a failure, while in the Shiga type of infection the bacilli disappeared from the stool on the second day of the disease. Morison, however, obtained encouraging results in an epidemic of dysentery; 70 cases were treated with 2 ccm of bacteriophage three times daily and of these three died and the rest were all cured. The author has treated a large number of cases of both acute and chronic dysentery with bacteriophage with indefinite results. In a few cases the effect produced appeared to be marvellous, but in the majority of cases no improvement could be detected. The results of trials by various observers, appear to indicate that bacteriophage therapy in dysentery is worthy of further trial.

Cholera. Bacteriophage therapy in cholera has been advocated. In this connection it is worthy of note that the therapeutic phage employed in the treatment of cholera must be virulent. The existence of a case with virulent bacteriophage should therefore draw special attention. Along with this must be considered the vibrios; some may yield to a phage of higher virulence while others may lead to actual diminution in the virulence of the bacteriophage. In order to obviate this difficulty Asheshov recommends a method of keeping therapeutic phage virulent by repeated cultivation on a freshly isolated vibrio.

The results published by d'Herelle and others (1930) who investigated the problem of bacteriophage on behalf of the Government of India appear to be striking enough. In a total of 198 cases of cholera, 74 received bacteriophage treatment, of whom only 6 died while in a series of 124 cases not receving the phage treatment the mortality rate was 58 per cent. In Brazil, as a result of controlled experiments conducted by da Costa Cruz (1923), the Brazilian Government have distributed bacteriophage for the treatment of bacillary dysentery. This mode of treatment quickly supplanted all others including the use of anti-dysentery serum, which has been abondoned. In Calcutta, dysenteryphage is being widely used. deMonte (1938) recorded the reports received from physicians who treated 90 cases with bacteriophage active against the important dysentery strains. Seventy six of these ninety cases were dysentery and good results were reported in 61 or 80 per cent; eight were diarrhœas and all recovered and six were colitis of which one benefitted. The dysentery phage was prepared in the School of Tropical Medicine and supplied in 2-3 ccm ampoules. The contents of one ampoule were taken in a wine glass of drinking water immediately after a dose of a mixture containing 60 grains of sodium sulphate and 10 grains of sodium bicarbonate. The number of doses administered varied; severe cases were given a dose every two hours up to six doses a day, mild and recovering cases one dose three times a day. Our experience with bacteriophage in the treatment of dysentery is that in frank cases of bacillary dysentery from which the infecting organisms have been isolated and identified, rapid and beneficial response may be obtained by the use of dysentery-phage; in chronic cases and in those patients from whom 'atypical flexner' organisms, Bact. pseudo-carolinus, etc., have been isolated, response to treatment with stock dysentery phage is uncertain and usually unsatisfactory.

In cholera, Morison and Vardon (1929) used a combined dysentery-cholera phage in two epidemics of cholera in Assam. A mortality of 75. 8 per cent resulted in cases having no bacteriophage, while the death rate was 29.0 per cent in cases receiving bacteriophage treatment. They mostly employed 2 ccm of phage four times daily by the mouth and serious cases received 2 ccm along with hypertonic saline. Asheshov (1931) treating cases claimed almost 100 per cent success. Souchard (1930) on the other hand failed to obtain any benefit and in his series a mortality of 24 out of 27 resulted. Others too obtained disappointing results. In a series 57 per cent died in the phage treated series whereas 53 per cent died in the control series.

More recently Pasricha, deMonte and O'Flynn (1936) in a carefully controlled experiment treated 398 bacteriologically proven cases of cholera with choleraphage and compared the results obtained with that obtained in 443 similar but alternate cases not given cholera phage but which in other respects received identical treatment. The case mortality in the phage treated groups was 8.3 per cent as against 17.8 per cent in the control series. Again Pasricha, deMonte and others (1939) conducted a comparative trial in which the results of treatment by five different methods (calomel, potassium permanganate, essential oils, choleraphage and M & B 693) were compared. Altogether 244 cases were treated and the results showed that cholera phage gave the best therapeutic results. Indeed these workers suggest that the result of choleraphage therapy are so encouraging as to justify the adoption of bacteriophage as a routine measure in the treatment of cholera. They administered choleraphage orally, about 2 ccm three to six times a day according to the acuteness of the attack in addition to hypertonic salines.

Enteric fever. Contrary to the favourable results obtained with bacteriophage therapy in the infections of the intestinal canal such as dysentery and cholera, its efficacy in enteric fever is doubtful. Some clinicians have reported excellent results. Strains of phage virulent

to the organism *in vitro* under laboratory conditions have been obtained, but these may be entirely ineffective in actual treatment of the disease. There are however,occasional favourable reports with bacteriophage treatment. The usual dosage in such cases in 2 ccm of the phage three times daily for four to five days. Intravenous injections of the phage have been resorted to, to lower the temperature and hasten recovery. In view of these results and of the diverse reports that have been published regarding bacteriophage therapy in typhoid and paratyphoid fevers, the question of application of bacteriophage in the treatment of this disease still remains unsettled.

Plague. The treatment of plague by bacteriophage has not as yet been carried out on a large scale and its value is therefore still undetermined. d'Herelle reported successful treatment of four cases of bubonic plague and stated that this method of treatment offers much promise. Couvy and his co-workers (1930 and 1932) reported favourable results of treatment in severe cases of human plague with a polyvalent phage. They affirmed the perfect safety of treatment of plague with bacteriophage even by intravenous injection. Two hours after injection of the phage, *Past. pestis* are found to be in a state of lysis. Pons (1932) reports very interesting results with bacteriophage therapy. He states that bacteriophage when given subcutaneously is rapidly generalised and produces lysis of the plague bacilli in 24 hours. As long as the lytic principles are present, blood or the aspirated material from the bubo remains sterile. Other workers have failed to substantiate these claims. Compton (1928) tried bacteriophage in experimental plague of animals but obtained indifferent results. Pirie (1929) stated that there is little evidence in support of either therapeutic or prophylactic value of bacteriophage in plague. Naidu and Avari (1932) had little success with a strain of powerful bacteriophage in human or in animal plague. Experiments on mice carried out in the Haffkine's Institute have shown that bacteriophage has no protective or curative effect in infection with *Past. pestis.*

Pyogenic infections. Certain favourable results are also reported in localised septic conditions due to staphylococci and streptococci. Furunculosis, carbuncles, abscesses, osteomyelitis and miscellaneous infections have been treated by this method. The usual procedure of application of bacteriophage in these cases is to administer it by subcutaneous injection, although a few investigators have combined such injections with the local application of bacteriophage in the form of moist dressings. Usually two injections, each of which may vary from 0.5 ccm to 3 ccm., have been given with an interval of from 24 to 48 hours. In the cases of *Bact. coli* infection of the bladder, this method is said to have given very encouraging results. Larkum (1926) demonstrated the existence of bacteriophage in most acute urinary infections and he later tried this method of treatment in suppurative conditions and found it satisfactory.

Certain general principles must be borne in mind for the clinical use of bacteriophage in these conditions. It must come into direct contact with the infected tissues and external application, direct injection and circum-injection are employed according to the type of lesions to be dealt with. Care should be taken during injections of bacteriophage inside a closed cavity, so that the tension is not thereby increased. It is, therefore, always safer to aspirate such pus as may be present in it, before injecting the phage.

Though local bacteriophage treatment by localised injections has given a certain measure of success, it is not at present possible to evaluate its importance in clinical practice. In these conditions there is direct contact between the invading organism and the therapeutic agent in the shape of bacteriophage, and it is not possible to say how far the results are due to bacteriophage therapy or other bacterial products that are present.

Summary.—About thirty years ago a curious phenomenon was first observed in laboratory cultures of bacteria. It was noted that in agar plates which had been thickly sown with colonies of staphylococci, clear areas which were found to be free of bacteria, had appeared. It was also observed that at a particular stage in the growth of certain cultures of the dysentery bacillus the entire culture would suddenly clear up and become free of bacteria. D'Herelle named the agent responsible for this phenomenon 'bacteriophage'.

It was surmised that if this bacteriophage agent could be made into a preparation which when administered to man produced its dissolving action on germs in the human body, it would be of great value in combating bacterial diseases. Intensive studies were, therefore, carried out in connection with bacteriophage. Bacteriologists and physicians cherished the hope of utilizing bacteriophage in the prophylaxis and treatment of diseases of bacterial origin.

Preparations of bacteriophage.—For therapeutic use bacteriophage is available in liquid form. It consists of a filtrate of broth cultures of bacteria which have been dissolved by the addition of bacteriophage. This filtrate, or lysate is used locally as a wet dressing or may be injected subcutaneously, intravenously or intraperitoneally to combat bacterial infections. It has also been given by mouth when its action is desired in the intestines, but unfortunately both gastric juice and bile have an inactivating effect on bacteriophage. When it is administered with sodium bicarbonate on an empty stomach, however, less seems to be destroyed and appreciable amounts get through into the intestine. Bacteriophage has also been administred in form of retention enemas. For local application the filtrate has been combined with a water-soluble jelly base to form a gel preparation.

Since each germ or at least each group of related germs is believed to produce a bacteriophage peculiar to itself, there are several filtrate preparations and several gel preparations. Bacteria from which these are made include the streptococcus, staphylococcus, *E. coli, V. cholerae* and such invaders of the respiratory tract as the pneumococcus, *N. catarrhalis*, etc. Frequently two or more types of bacteria are combined in one preparation.

Nature of bacteriophage.—D'Herelle thought that the causative agent was a living substance, too small to be seen by the most powerful microscopes. The bacteriophage was considered to be parasitic on bacteria which it destroyed in the process of reproducing itself; in fact it was an ultra-microscopic parasite on a microscopic parasite.

Opposed to this view is the belief that bacteriophage is a by-product of the metabolism of the bacteria themselves. According to this concept the staphylococci or dysentery germs produce a substance which is toxic to the germs themselves. The effect produced by bacteriophage is, from this view-point, an autointoxication and there is no question of its being an external organism of parasitic nature.

This latter view has been strengthened by recent studies on the behaviour and chemical nature of bacteriophage. Chemically, bacteriophage has been shown to be a protein of high molecular weight. It forms only when conditions are favourable for the growth of bacteria, and the better the bacteria grow the faster is the bacteriophage produced. Under ideal laboratory conditions the following sequel of events takes place: bacteria multiply rapidly and continuously; bacteriophage begins to form and increases in quantity; a point is reached where enough bacteriophage has been produced to lyse all of bacteria present and this is rapidly accomplished.

The question arises why the phage does not form in the body when bacteria have invaded the body and are freely multiplying thus freeing the body of the infection. Further laboratory studies have shown that bacteriophage is inactivated by a variety of substances amongst which are blood serum, white blood cells. tissue debris such as dead cells, and many other things which are found in the body where germs are growing. It is for this reason that the dissolving action on bacteria, seen so rapidly in test tubes where no serum or tissue is present, is prevented in the body.

Mode of action of phage in vivo.—In spite of its inactivation within the body there are some situations in which bacteriophage may be of value, and attempts have been made to learn how it produces its action in the body. Four possible ways have been suggested to explain the mechanism of phage activity in the body and at least three of these are of unquestionable importance.

Firstly, bacteriophage stimulates the activity of phagocytic cells of the blood and tissues. These "scavenger cells" are of tremendous importance in freeing the body of certain kinds of germs, and their activity in the presence of large amounts of bacteriophage is considerably increased and they are thus enabled to engulf and destroy the bacteria. For example in the case of boils, the "scavenger cells" engulf tremendous numbers of the infecting bacteria and the use of the appropriate phage gel locally on the boil to stimulate this activity is beneficial.

Secondly, when bacteria are dissolved by phage in the body the components of the lysed bacteria act as antigens to stimulate the formation of antibodies. These antigenic actions are greater than those of undissolved bacteria and so indirectly phage increases immunity to the diseases and acts as a vaccine. When the bacteriophage is administered as a biologic preparation, the benefit actuallv comes from the bacterial parts that have been liberated when phage was added to the bacterial culture.

Thirdly, phage preparations may activate non-specific mechanisms of immunity in the body, comparable to those stimulated by other non-specific vaccines. In certain cases a non-related substance may promote immunity to attack from a particular germ and there is sufficient evidence to indicate that phage lysates may sometimes act in this fashion.

Fourthly, it is possible, but not very probable that phage may affect growth of bacteria in the body in such a way that less virulent forms develop.

Value in therapy.—Had it not been for spectacular development of the sulphonamides and antibiotics, bacteriophage therapy might be in greater use today than it actually is. Some observers have obtained good results in the prevention and treatment of cholera with bacteriophage preparations. Properly prepared lysate can be useful as a vaccine for certain infections caused by staphylococcus such as boils, as an agent to induce non-specific protein shock in certain diseases such as typhoid fever and as a local agent to stimulate the activity of the "scavenger cells" in a locally infected area. Many promising possibilities were anticipated. With the chemotherapeutic and antibiotic agents now available phage therapy has been relegated to the background. It is possible that further research may show that phage has a definite place in the treatment of bacterial diseases.

CHAPTER II

BACTERIAL DISEASES—SULPHONAMIDES

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SULPHONAMIDES *

1. Introductory

Introductory.

Chemotherapy of infections with bacterial organisms has been developed along two lines, (*a*) the employment of chemical compounds whose antibacterial properties are relatively high as compared with their toxicity for animal tissues; (*b*) antibiotic substances, produced from molds, bacteria, etc.

For a substance to be successful in killing bacteria in the circulating blood, two essential conditions must be fulfilled. It should, after administration, attain a concentration in the blood lethal to the microbes but at the same time be harmless to the host, and it must maintain such a concentration sufficiently long for the microbes to be killed.

If a germicide is introduced into the circulation, the concentration of the drug will be at its maximum immediately after the injection, and simultaneously with it, the bactericidal power will also be at the highest. If, on the other hand, there is no increased bactericidal power of the blood immediately after the injections, but only after a lapse of time, such an increase cannot be ascribed to the drug alone, but to some other factor. This is significant from the fact that normal human blood has got a great bactericidal property. It has been found that 1 ccm of human blood when incubated with 2,000 to 5,000 staphylococci can destroy 90 per cent of the cocci. When chemotherapeutic substances are injected into the blood the reaction that takes place is complex. These agents may act directly on the organisms so as to cause their death (bactericidal) or they may inhibit the growth of the bacteria (bacteriostatic); or, their action may

*In preparation of this Section I gratefully acknowledge the help received from Medical Research Council War Memorandum No. 10. *The Medical use of Sulphonamides,* (1945) and "Sulphonamides in Theory and Practice" by J. Stewart Lawrence (1946) H. K. Lewis and Co. Ltd., London.

be an indirect one due to stimulation of the natural defensive mechanism of the tissues. All three of these factors may come into play. If the resistence of the invading organism is lowered by direct action, then the cellular and humoral tissue defences may be enabled to come into play. Further the products resulting from the destruction of the organisms act as antigens, and in this way immunity reactions are developed.

Of the chemical compounds used to combat bacterial and virus diseases, the group of sulphonamides are most effective.

Historical and general.—The search for a specific chemical substance for the treatment of microbic infections has been conducted from the early days of bacteriology. The first great advance in chemotherapy was made when Ehrlich introduced salvarsan (606) for the treatment of syphilis. Later work in this field produced a number of organo-metallic compounds of arsenic and antimony which have remarkable curative properties against diseases due to protozoal and spiral organisms. For nearly two decades after the discovery of organic arsenicals, although improvements were effected in the treatment of protozoal infections, no advance was made in combating the large group of bacterial diseases. Most of the chemical compounds prepared as chemotherapeutic agents were used in the treatment of malaria, leishmaniasis, trypanosomiasis, amoebic dysentery, etc. Attempts to apply chemotherapeutic principals to bacterial diseases were unsuccessful, for most of the antiseptics and disinfectants which were then known, have no specific action on the microbes in preference to their effect on the tissues of the host in which they are embeded. Further most of these drugs are general protoplasmic poisons and destruction of the septic organism in a wound surface entails the destruction of the surrounding tissue cells also. It is, therefore, very difficult to disinfect the infected tissue because a drug circulating in the blood in sufficient concentration to destroy the invading bacteria will be equally detrimental to the organs in which they are embedded. Many unsuccessful attempts were made in the quest of a substance which, when introduced into the body, would differentiate between the protoplasm of the bacteria on which it would act leaving the tissues of the host unaffected. The sterilization of the blood stream against some bacterial infections was looked upon as an unattainable ideal till recently. It was, therefore, an event of a far-reaching importance when Domagk (1932) embarked upon a new epoch in the history of chemotherapy, and showed that the azo dye patented under the name of "Prontosil", administered to mice, could cure them of septicaemia experimentally produced by injecting haemolytic streptococci.

Then for the first time in the history of infectious diseases, a synthetic chemical compound was produced which was therapeutically effective against a bacterial infection in animals and man. Even more surprising was the fact that this group of drugs was ripe with possibilities in respect of this effect on a large number of bacterial infections.

Prontosil and allied compounds.

Azo dyes.—The discovery of the anti-bacterial effects of sulphonamide is looked upon as one of the greatest therapeutic discoveries in modern medicine. Although Gelmo had first described para-amino-benzene sulphonamide (sulphanilamide) in 1908, its antibacterial properties were not appreciated till 1935. Horlein, Dressel and Kothi, working on azo textile dyes, prepared the first azo dyes containing sulphonamide in 1909 and found that they were characterised by greater fastness to washing and milling and one of them possessed a limited therapeutic action against mouse septicaemia caused by haemolytic streptococci. The bactericidal action of certain azo compounds in vitro was later demonstrated and chrysoidine was used for chemotherapeutic purposes but not the slightest

therapeutic effect could be obtained in infected animals. Further work led to the discovery of azo compounds with marked bactericidal properties *in vitro* but no action *in vivo* in the mouse. Later studies confined to compounds containing the sulphonamide group and tested only by animal experiments, led to the discovery of drugs effective against streptococcus infections in animals. In 1932 a patent was obtained for the azo dye sulphonamide-crysoidin which was found by Domagk (1935) to cure an otherwise fatal streptococcus infection in mice. This compound which was very sparingly soluble was placed on the market under the trade name of "Prontosil" (Prontosil rubrum) and secured for Domagk the Nobel Prize. A more soluble compound for intramuscular injection was described shortly afterwards and was called "Prontosil soluble" or "Neoprontosil". In France a similar compound named "Rubiazol" was prepared in 1935 and the experimental work of Domagk was confirmed. It was also shown (1936) that in the tissues of the host the azo dyes were split at the azo linkage and that prontosil yielded para-amino-benzene sulphonamide which was thought to be the chemotherapeutic portion of the molecule. This compound was prepared by Fourneau and it was shown that it was as effective in curing experimental infections as was prontosil.

Even before the appearance of Domagk's paper in 1935, mention of the treatment with "Streptozon" (Prontosil) of patients with infections appeared in German clinical journals and afterwards numerous favourable results in human infections were reported. It was only, however, after the publication of the paper on the marked beneficial effect of prontosil in puerperal sepsis that considerable interest was awakened in the English speaking countries with regard to these compounds.

Buttle and others took up the work and confirmed the findings that para-amino benzene sulphonamide was effective in haemolytic streptococcal infections in mice, and also cured meningococcal infections. Work was also started in the U. S. A. which confirmed the previous work with regard to para-amino benzene sulphonamide and further basic contributions were made with regard to its pharmacology and toxicology. The name of sulphanilamide was given to this compound by the Council of Pharmacy and Chemistry of the American Medical Association. This work led to the extension of use of sulphanilamide in the chemotherapy of infections other than those with hæmolytic streptococci. A large number of derivatives of sulphanilamide were developed and tested for their clinical value not only in bacterial infections but also in protozoal and virus infections. Some of these compounds are benzyl sulphanilamide (septazine), di-sulphanilamide (disulon) and its dimethyl derivative (uleron). The best of these compounds was sulphapyridine (M & B 693) synthesized in England.

Newer compounds.—Whitby (1938) working with sulphapyridine on mice inoculated with pneumococci and with haemolytic streptococci, found that it appeared to exert an action on the capsule of the pneumococcus and that it was as active as sulphanilamide against a haemolytic streptococci and meningococci. Many new compounds were produced and a flood of communications on the subject has been appearing ever since. Further work has greatly clarified the views on the whole of this group of drugs.

Sulphathiazole and sulphamethylthiazole were introduced in 1939 with high hopes for activity against staphylococcal, pneumococcal and other infections and encouraging results were also obtained against plague in India. New compounds, such as sulphanilyguanidine or sulphaguanidine, sulphadiazine, sulphamerazine, etc., have been prepared which are more effective and less toxic. Sulphaguanidine

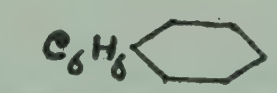
is little absorbed from the gastro-intestinal tract and has given good results in acute bacillary dysentery and cholera and sulphadiazine and sulphamerazine are reported to be less toxic than sulphathiazole and sulphapyridine. Many new uses and methods for application of these compounds are developing, particularly in surgery.

During recent years the number of sulphonamide compounds have rapidly multiplied and are being tried in all sorts of conditions and diseases. War Memorandum No. 10 of Medical Research Council of England summarised the position with regard to this group of compounds for the benefit and guidance of Military Medical Officers and has been freely utilised in the preparation of this section.

Although these compounds have been recently overshadowed by the rapidly developing group of antibiotics, there is no doubt that their reliability in the treatment of many infections is still great. Their proper use, however, needs a thorough comprehension of their pharmacological action, therapeutic application and toxic effects. These have therefore been dealt with in detail.

2. Chemistry and nomenclature

Term Sulphonamide.—On account of their complicated chemical structure and partly because of the confusion that has resulted from the variety of trade names used by different commercial firms to designate almost identical sulphonamide compounds, a certain amount of confusion has been produced in the mind of medical practitioners. The essential chemistry of these compounds is not difficult to understand. The term 'sulphonamide' is used in chemistry to designate the amide of sulphonic acid and in medicine is used to include also the substituted sulphonamides. The first sulphonamide used in therapeutics by Domagk (1935) was sulphonamido-crysoidin or Prontosil rubrum against streptococcal septicaemia in mice. This compound is an azo dye which liberates sulphanilamide in the body, to which most of the activity is attributed. Sulphanilamide (para-amino-benzene sulphonamide) itself is the simplest of the sulphonamides and is the mother substance of the other derivatives. This substance has been known to organic chemists for nearly 40 years, is soluble in water, colourless and comparatively slightly toxic for mammals. It is the therapeutically active principle of the azo compounds and sulphanilamide and its derivatives have largely replaced them in therapy.

All these compounds contain at least one benzene group,  at least one amino group (NH_2) and at least one sulphonamide group (NH_2SO_2). If we start with benzene and add amino group (NH_2) to it we obtain amino-benzene  which is the chemical name for well known substance aniline. By attaching a sulphonamide group to the amino-benzene in the para position we obtain para-aminobenzene sulphonamide.

 or aniline sulphonamide which has been abbreviated to sulphanilamide and so the simplest of all such compounds is formed. The entire series of these remedies is known as the sulphonamides, because they contain the sulphonamide group, *viz.*, $-SO_2NH_2$.

Two classes are recognized:—

Acetylation of the sulphonamide group yields sulphacetamide, while substitution of the amido group with the pyridine, thiazole, pyrimidine and dimethyl pyrimidine radicles yields respectively sulphapyridine, sulphathiazole, sulphadiazine and sulphadimethyl pyrimidine. In sulphaguanidine the amido group of sulphanilamide is replaced by the guanidine radicle. All these compounds form white or faintly yellowish white crystalline powders, which stand heating from 140 to 150°C but decompose at higher temperatures. They are sparingly soluble in water but more soluble in mineral acids, and (with the exception of sulphaguanidine) in aqueous solutions of alkalies. Sulphacetamide, sulphapyridine, sulphathiazole and sulphadiazine are available in the form of sodium derivatives, which form white powders, readily soluble in water.

Nomenclature.—Buttle (1939) has published a simple classification of the drugs now in general use based on the fact that sulphanilamide consists of a benzene ring to the opposite ends of which are attached an amino and a sulphonamide group. The derivatives of sulphanilamide can be divided into two groups:—those in which the substituents are introduced into the amino group, and those in which the substituents are introduced into the sulphonamide or amido group.

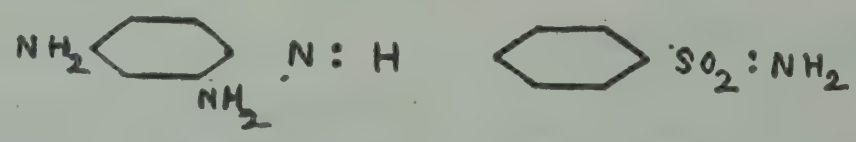
Group A.—The first group comprises sulphanilamide itself and those compounds which contain the sulphanilamide radicle and in which the amino group of the benzene ring is substituted by other groups. The first group includes prontosil (album and rubrum and rubrum and soluble), proseptasine, soluseptasine and rubiazole, streptocide, prontyline, sulphonamide, preparation 5214 and others.

Group B.—The second group includes sulphapyridine, sulphathiazole, sulphadiazine, sulphamezathine, sulphaguanidine, etc. In these the amido group is substituted by other groups such as pyridine, thiazole, guanidine, etc.

3. Sulphonamide drugs

Sulphonamido-crysoidin.

(1) *Sulphonamido-crysoidin,* 4-sulphonamide-2': 4'-diaminoazobenzene. ('Prontosil red', 'Prontosil rubrum or flavum'),

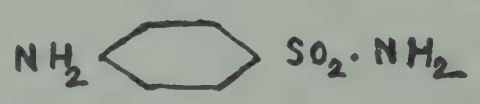


Almost insoluble in water, readily soluble in acetone. This was the first sulphonamide compound to be used in therapeutics. It is an azo-dye and in the body it liberates sulphanilamide to which its activity is due. It is now replaced by the latter drug.

Azosulphamide ("Bayer 102", Prontosil S or II, "Neoprontosil", Rubiazol injectible) is a soluble derivative.

sulphanilamide.

(2) Sulphanilamide (B.P.;U.S.P.) p. aminobenzene sulphonamide.



Solubility in water, at 15.50°C, 1 in 250; at 20°C, 1 in 170; at 25°C. 1 in 115. More soluble in presence of alkali, but no sodium compound is available in solid form. Soluble in acetone, sparingly soluble or insoluble in other common organic solvents. Melting points 164.5-165.5°C.

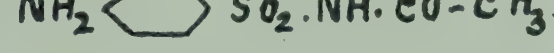
Sulphanilamide is rapidly absorbed from the gut, and with the dosage usually employed, *viz.,* an initial dose of 4 gm followed by 1 gm every 4 hours, a blood concentration of 5 mgm per cent is maintained and this is considered suitable for moderately severe infections. Absorption from the peritoneal cavity and from denuded areas of extensive burns is also quite rapid, but in the case of wounds, absorption is slower and irregular and in these cases if there is co-existing systemic infection, local applications of the drug require to be supplemented by oral therapy. The drug may also be administered by the intravenous or subcutaneous routes in the form of a 0.8 per cent solution in saline or glucose. The rectal route may also be used. but twice the oral dose is necessary to maintain adequate blood concentrations. In severe infections it may be desirable to raise the blood concentrations to 10 mgm per cent and this can be achieved by doubling the dosage employed for infections of moderate severity. In plasma 5 per cent of the drug is combined with proteins and 10 to 15 per cent is acetylated, so that nearly all is in active form; 90 to 95 per cent of the dose administered by mouth is excreted in urine, 2 to 3 days being required for complete elimination of the drug after it is discontinued.

The drug is concentrated 10 to 20 times in the kidney and excreted entirely by glomerular filtration and three quarters is re-absorbed in the tubules. The amount excreted thus varies with the output of urine. When the drug is applied to a wound only about half appears in urine, the rest being probably destroyed or exuded from the wound.

The toxicity of the drug is not high, agranulocytosis is rare, but acute haemolytic anaemia and acidosis are peculiar to this drug. Nausea, vomiting, headache and drug fever commonly occur and drug eruptions on parts exposed to light are not uncommon. Renal complications practically never occur. Necrosis of liver rarely occurs and it may be fatal.

Sulphanilamide is particularly suited for local application because on account of its solubility and diffusibility no concretions form. It is often combined with sulphathiazole to prolong its local action. For systemic use the drug is not suited on account of its low potency.

(3) *Sulphacetamide* (*B.P.C.*), p-amino benzene sulphonacetamide. ("Albucid" "Sulphacetamide"). *Sulphacetamide.*



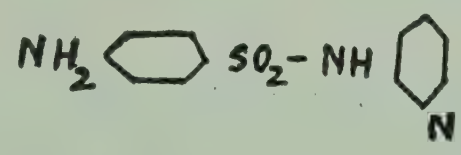
Solubility in water, at 20°C, 1 in 150. Soluble in alcohol and in acetone, insoluble in ether. Melting point 181-184°C.

Sodium compound (Soluble sulphacetamide B.P.)-Solubility in water, 1 in 1.5; sparingly soluble in alcohol and in acetone. In contrast to solutions of the sodium salts of most other sulphonamides, which are strongly alkaline, aqueous solutions of sodium sulphacetamide are only feebly alkaline, and they may be made neutral without precipitation of the compound. They are, therefore, useful for local application to delicate tissues, such as the conjunctiva.

Sulphacetamide is more powerful than sulphanilamide (Sulphanilamide equivalent of 8). A dose of 4 gm followed by 1 gm every four hours produces a concentration of 7-10 mgm per 100 ccm in the blood. It is useful in *B. coli* infections of the urinary tract. A 30 per cent solution of the sodium salt when applied to the conjunctiva locally penetrates the cornea and gives moderate concentration in the aqueous humour and iris; for local action a 10 per cent solution is effective. It penetrates the skin more effectively than other sulphonamide drugs reaching concentrations of 20 mgm per cent in the dermis. The sterile powder is suitable for application to wounds and burns or to the peritoneum.

The drug may produce nausea, vomiting and cyanosis, but toxic effects are not so marked as those of sulphapyridine or sulphanilamide.

(4) *Sulphapyridine (B.P.C.,U.S.P)* 2: p-amino-benzensulphonamido-pyridine. ("M. & B. 693", "Dagenan").



Solubility in water is low; at 20°C, 1 in 3,000; at 100°C, 1 in 100. Soluble in alcohol and in acetone. Melting point, 191-193°C. Sodium compound-solubility in water, at 15.5°C, about 1 in 3: in alcohol at 15.5°C, about 1 in 20.

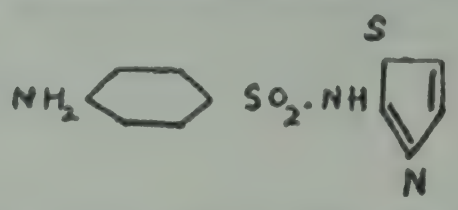
Absorption rate is slower than that of sulphanilamide and is erratic; after a single dose maximum concentration is not reached till after 5 to 6 hours. For infections of average intensity, an initial dose of 4 gm followed by 1 gm every 4 hours, maintains an average blood level of 5 mgm per cent. The sodium salt is readily soluble and is used for intravenous or intramuscular injections. The latter route should be avoided if possible as the high alkalinity of the solution (pH about 10) is likely to produce necrosis. The sodium salt may also be given subcutaneously as a 0.5 per cent solution, or by rectal drip as a 2 per cent solution, 250 ccm as an initial dose followed by 125 ccm every four hours. In severe infections the maintainance of a blood level of 10 mgm per cent has been recommended. It must be borne in mind that sulphapyridine has a definite antipyretic action, and the temperature may come down without the infective process having been controlled. In plasma much of the drug occurs in conjugated form; only 10 per cent is combined with plasma protein. Concentration in red cells is 60 per cent of that in plasma and up to 80 per cent of plasma concentrations are obtained in the cerebrospinal fluid. The drug is concentrated in the liver to double the blood level.

Sulphapyridine is a toxic drug and frequently produces toxic reactions especially nausea, vomiting, headache, depression and mental confusion in the majority of patients. Some degree of cyanosis is common and drug fever and rash are not uncommon. Hæmaturia, anuria and agranulocytosis and hepatitis are other undesirable effects occasionally met with. Owing to marked toxic effects the sphere of utility of this drug is limited, has however, proved useful in the treatment of dysentery, due probably to its slow rate of absorption from the gut and preventing the utilisation of p-aminobenzoic acid by the bacteria.

Renal clearance of this drug is low. Only 30 to 60 per cent of the amount taken appears in urine and more than 50 per cent in acetylated form, which has low solubility (11-89 mgm per cent) at pH of urine (5.4 to 8.2). Excretion is not completed even in 72 hours. Amount occurring in the fæces is variable but may be 20 per cent or more of the amount taken.

Sulphathiazole.

(5) *Sulphathiazole* (B.P.C.: U.S.P.) -2-p-aminobenzenesulphonamide-thiazole (Ciba 3714 or "Cibazol", "M. & B. 760" Thiazamide, "Eleudron").



Solubility in water, at 15.5°C, about 1 in 2,500, is greater than sulphapyridine; 96 mgm per cent in water and 250 mgm per cent in serum and 190-900 mgm per cent in urine. Slightly soluble in alcohol. Melting point 200 to 203°C. Sodium compound-solubility in water, at 15.5°C., about 1 in 3; in alcohol, at 15.5°C., about 1 in 20.

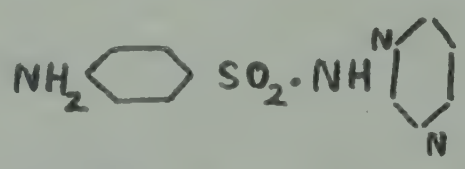
Sulphathiazole has probably the most potent bacteriostatic action of all the sulphonamide compounds, its sulphanilamide equivalent being 50, and its bacteriostatic constant 1/53 for *Staph. aureus.* It is rapidly absorbed from the gut, and the blood level reaches its maximum in 1 to 4 hours after a single dose. To maintain a blood level of 5 mgm per cent, an initial dose of 4 gm followed by 1.5 gm every 4 hours is required. The sodium salt is very soluble (1:3) and is suitable for intravenous use, or for subcutaneous use as a 1 per cent solution. In very severe infections, it is advisable to maintain a blood level of 7 mgm per cent, and for this, the ordinary dosage should be increased by 50 per cent.

The toxicity of sulphathiazole is considerably less than that of sulphapyridine, nausea, vomiting and cyanosis being common. Drug fever accompanied sometimes by enlargement of spleen and lymph glands and rashes are common, especially after the seventh day of administration and during the second or subsequent courses. Transient arthritis and also conjunctivitis of a nodular form may occur. It is, therefore, advisable to use another sulphonamide preparation, should a second course be required. The chief risk to be feared with sulphathiazole, is that of hæmaturia, oliguria and sometimes anuria, due to deposition of acetyl sulphathiazole crystals in the renal tubules and ureters. The liver is only rarely affected. Agranulocytosis, thrombopœnia, aplastic anæmia and purpura sometime occur. Local application of sulphathiazole to the skin may produce a sensitisation dermatitis which is liable to recur.

Due to its great bacteriostatic powers, this drug is indicated in all severe infections. It may also be used in infections with organisms of low drug sensitivity. The drug is, however, toxic and should only be used when less toxic drugs will not serve the purpose. It is contraindicated in cases with dehydration and diminished urine secretion. It has been found very useful in local applications to wounds in powder or micro-crystalline form on account of its solubility and slow absorption.

Sulphadiazine.

(6) *Sulphadiazine* 2-p-aminobenzenesulphonamide-pyrimidine (Pyrimal). It has a sulphanilamide equivalent of 36. Solubility in water at 25°C., about 1 in 13,000 (15 mgm per cent) sparingly soluble in alcohol and in acetone. In serum solubility is 160 mgm per cent, in urine 18-52 mgm per cent at body temperature. Melting point, 252-256°C. Sodium compound has a solubility in water, at 25°C. 1 in 2 and pH of 9.2; sparingly soluble in alcohol.



Sulphadiazine is a less powerful bacteriostatic drug than sulphathiazole. It is slowly but completely absorbed from the gut, maximum blood concentrations being reached in 3 to 36 hours, but usually within 6 hours. Generally blood levels of 10 mgm per cent are required to be maintained and are achieved by an initial dose of 4 gm followed by 1 gm 4-hourly for the first day and 1 gm 6-hourly subsequently. Due to its slow absorption, it is advisable to give the initial dose of the drug intravenously in all severe or moderately severe infections, and thereafter continue with oral medication. If subsequent doses are also given intravenously, the dose should be 3 gm every 12 hours. By the subcutaneous route 0.3-1 per cent solution in saline is given.

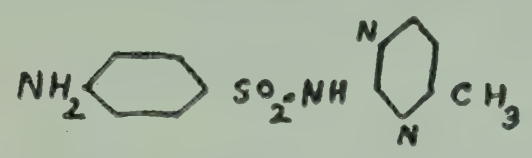
Sulphadiazine has a low renal clearance and this enables high blood concentrations to be easily maintained. High concentrations in blood are obtained in moderate renal impairment and if these do not exceed 20 mgm per cent no harm results; usually this should not exceed 15 mgm per cent. The drug is acetylated in the plasma to the extent of 5-10 per cent, which is proportionately lower than other sulphonamides. At the normal sulphadiazine and protein levels of blood, about 20 per cent of the drug is bound to protein and as a result it easily diffuses into exudates and body fluids. In the cerebro-spinal fluid the concentration is 50 to 80 per cent of that in the blood.

Only about 65 per cent of the intake is excreted in urine in 3 to 4 days and about one third is conjugated. Sulphadiazine is one of the least toxic drugs of the sulphonamide series and serious toxic reactions such as agranulocytosis and purpura are rare. Nausea and vomiting only occur in a small percentage of cases. Its low solubility in the urine, however, frequently causes haematuria and anuria, especially if alkalis are not given concurently. The low toxicity of the drug makes it the drug of choice for all moderate and mild infections, and for prophylactic use in ambulant patients. The drug is contra-indicated in all cases with any degree of renal impairment.

The drug is suitable for local application especially to corneal ulcers, burns, etc., but on account of its low solubility it is dissolved in 8 per cent triethanolamine as a 2.5 per cent solution.

It is suitable for use against all infections with sulphonamide susceptible organisms and in similar doses is more effective than sulphathiazole.

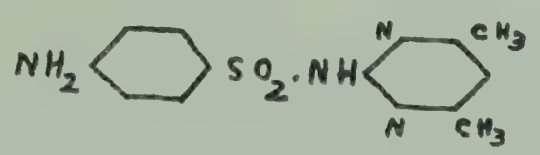
(7) *Sulphamerazine*, 2-sulphonamido-4-methylpyrimidine, is a methyl derivative of sulphadiazine. It is also described as "Sulphamerazine" or Sulphamerizine. Its sulphanilamide coefficient is about 20, and its therapeutic activity and toxicity are similar to *Sulphamerazine.*



sulphadiazine. Solubility 24 mgm per 100 ccm in water at 20°C. In the plasma 80 per cent of the drug is combined with protein and only 20 per cent is free. For this reason, concentration in the tissues is low. About 70 per cent of intake appears in urine slowly. Concentrations of more than 25 mgm per cent in blood have been maintained in monkeys for several days without ill-effects provided fluid intake is adequate. The acetyl derivative and the drug itself are both more soluble than sulphadiazine in urine; its fæcal concentration is lower than sulphadiazine. It is more slowly excreted than the other sulphonamides, and an adequate concentration in blood (8 mgm per cent) can be maintained by an initial dose of 4 gm, followed by 1 gm every 6 hours. It is not effective by the rectal route.

Sulphamerazine is less toxic than either sulphanilamide, sulphapyridine or sulphathiazole, but the incidence of renal complications, drug fever, rashes, etc., is greater than with sulphadiazine. The slow excretion makes it possible to maintain adequate blood levels with small and infrequent doses, and for this reason the drug is particularly useful for prophylactic purposes, and used in this way, toxic effects are slight and uncommon.

(8) *Sulphamezathine.* 2-(4-p-aminobenzensulphonamido)-4:6-dimethylpyrimidine is a dimethyl derivative of sulphadiazine. ("Sulphamezathine" is a trade name). Its sulphanilamide coefficient is 13. *Sulphamezathine.*



Slightly soluble in water, about 1 in 3,000 (190 mgm per cent), and 191-297 mgm per cent in urine at body temperature; sparingly soluble in cold alcohol, and moderately soluble in hot alcohol. Melting point 197°C, no decomposition during heating at 150-160°C for 30 minutes. Its potency is more or less the same as other compounds of sulphadiazine series though it is said to be more potent against pneumococcal infections.

This compound is rapidly absorbed from the alimentary canal, but less readily excreted; a high concentration in the blood (9 mgm per cent) can thus be maintained by such moderate doses as 4 gm initially followed by 1 gm 4 hourly. It is advisable to maintain a level of 10 mgm per cent in severe infections. The sodium salt is quite soluble (1 in 3 at 15°C), and is suitable for intravenous use. Three grams of this salt intravenously give a blood level of 11 mgm per cent at 2 hours and 9 mgm per cent at 4 hours. For subcutaneous administration 0.5 per cent solution in saline is used. In plasma 38 per cent of this drug is conjugated and 80 per cent of the unconjugated drug is bound to protein. It, therefore, diffuses less readily into tissues than sulphadiazine.

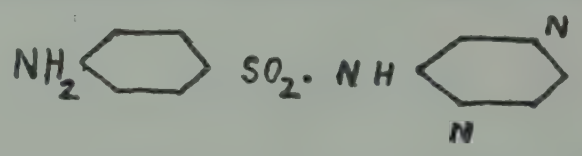
Sulphamezathine is the least toxic of the sulphonamides, and this fact combined with its comparatively high potency make it a most suitable drug for routine use. It is said to be particularly indicated in pneumococcal infections of all grades of severity. It does not diffuse readily into the spinal fluid; sulphadiazine is, therefore, to be preferred for meningitic infections. The rapid excretion and ready solubility of the drug in the urine (115 to 176 mgm per cent) make it particularly suitable for use in the treatment of infections of the urinary tract. Crystals do not form in urine and between 70 and 90 per cent of the drug can be recovered from the urine.

Sulphamethazine is the least toxic of the sulphonamide drugs, vomiting, cyanosis and headache being present in 1.1 per cent of cases and rashes and drug fever only rarely. No cases of blood and renal damage have been met with.

This drug is most suitable for routine use in all infections particularly pneumococcal in origin on account of its high potency and low toxicity. It is not likely to be useful in intestinal infection owing to its rapid and complete absorption nor is it as suitable as sulphadiazine in meningitis because of its low diffusibility into the cerebro-spinal fluid.

Sulphapyrazine.

(9) *Sulphapyrazine,* 2-sulphanilamidopyrazine. Has a sulphanilamide coefficient of 50.

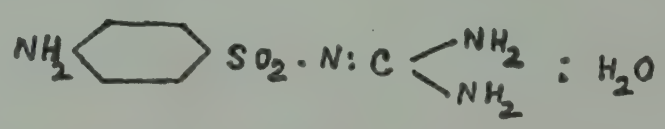


Absorption from the intestine is moderately slow, maximum blood concentrations of 5 mgm per cent being reached in 4 to 6 hours. The drug is as active in vitro against *B. coli* and pneumococci as sulphathiazole. In experimental animal infections, it proved as active as sulphathiazole against streptococci and pneumococci and as sulphadiazine against staphylococcal infections. It is largely excreted in fæces and concentrations like sulphaguanidine can be obtained with half the dose.

Sulphapyrazine has not yet been sufficiently employed in clinical practice for adequate evaluation of its efficacy. Reports, however, indicate that the drug may prove more effective in the treatment of bowel infections, such as dysentery, especially in the carrier stage, than any of the other sulphonamide compounds.

Sulphaguanidine.

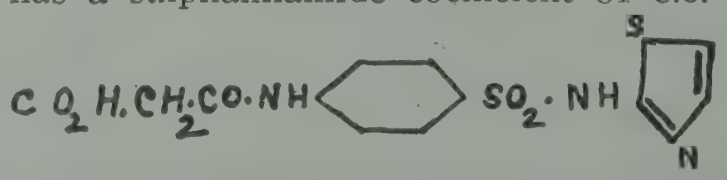
(10) *Sulphaguanidine* (B.P.; U.S.P. Sulphanilylguanidine)-p-aminobenzenesulphonylguanidine is also described as Guamide.



Solubility in water at 25°C about 1 in 1,000, at 100°C about 1 in 10. Sparingly soluble in alcohol and in acetone. Insoluble in aqueous solutions of sodium hydroxide. Melting point, 190-192°C. This compound is incompletely absorbed from the gastro-intestinal tract, and it is, therefore, useful for the treatment of bacillary dysentery and similar infections. A dose of 6.0 gm followed by 3.0 gm every 4 hours prescribed in adults produces a blood level of 5.0 mgm per 100 ccm, less if diarrhœa is present. The dose may be reduced to 3.0 gm 8 hourly when diarrhœa has ceased. In plasma 8 per cent is combined with proteins and 40 per cent is acetylated. Diffusions into the tissues occurs in the same concentrations as in blood. It is rapidly excreted in the urine (15 to 53 per cent of intake) but crystals occur in urine frequently. A concentration of 1 per cent in the fæces for 6-9 days reduces the number of *B. coli* in the stool. For this reason it is used to reduce bacterial contents in surgical operation of the gut and in the treatment of bacillary dysentery. Concentrations of 400 to 4,000 mgm per cent are found in the fæces after an oral dose of 6 gm. Toxic symptoms are slight and infrequent; occasional nausea and vomiting, headache, drug fever, dermatitis or conjunctivitis may occur. Rarely agranulocytosis, hæmaturia and oliguria may occur.

(11) *Irgafen*-N1-3-4 dimethyl benzoyl-sulphanilamide has a sulphanilamide coefficient of 50. It is effective against hæmolytic streptococci, pneumococci and staphylococci but has no action against *B. coli.* It is excreted more slowly than sulphadiazine and thus produces a high blood level. This compound has not yet been tried on a large scale.

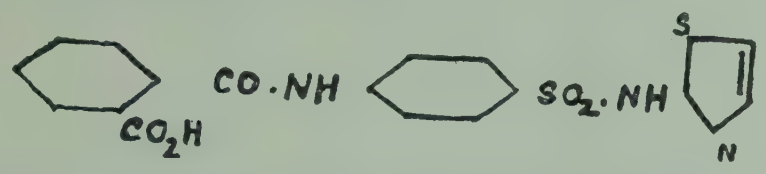
(12) *Succinylsulphathiazole-2-* (p-succinyl-aminobenzenesulphonamido)-thiazole ("sulphasuxidine"; Colistatin); has a sulphanilamide coefficient of 0.6.



It is soluble in alcohol, acetone and sodium bicarbonate solution, but very insoluble in water. It is poorly absorbed in the intestine, only about 5 per cent of the ingested drug being excreted by the kidneys, and the concentration in the blood is always low. Its action is principally on the coliform organisms in the lumen of the bowel, which become greatly diminished in number. There is evidence that this effect is due to the liberation of small amounts of sulphathiazole. Succinylsulphathiazole has been used mainly for the treatment of dysentery and in the preparation of patients for operations on the large intestine. It is less toxic than sulphaguanidine and as effective in the treatment of bacillary

dysentery; doses of 6 gm 4 hourly for the first day and 3 gm 4 hourly thereafter produce a concentration of 85-200 mgm of free sulphathiazole per 100 ccm of fæces. Fewer toxic reactions than sulphaguanidine are encountered.

(13) *Phthalyl-sulphathiazole-* 2-(p-phthalylaminobenzenesulphonamido)-thiazole, also known as sulphathalidine closely resembles succinylsulphathiazole. It is almost insoluble in water, but its sodium salt is soluble.



The absorption is even less than that of succinyl-sulphathiazole, and is said to be non-toxic by mouth. It is effective against coliform organisms in the dog's intestine and has been found to be effective in the treatment of bacillary dysentery in man.

Marfanil.

(14) *Marfanil* (*Homosulphanilamide*), is 4-aminomethylbenzenosulphonamide or p-sulphonamidobenzylamine. Marfanil is the German trade name and it is known as sulphabenzamine and sulphamylon. It is freely soluble in water. It has been widely used in



Germany and elsewhere for local application to wounds. Its action differs from other sulphonamides, in that it is not abolished by p-aminobenzoic acid or by the presence of pus or necrotic tissue. The drug is more effective against anaerobic organisms, particularly *Cl. welchii;* no action on *B. coli.* It is excreted too rapidly and is thus not suitable for internal use. It is used against sulphonamide-resistant streptococci.

(15) *Sulphanilylbenzamide* (M. P. 181°C.—182°C.) was found to be the best bacteriostatic agent next to Sulphathiazole. It is absolutely free from undue toxicity, fairly absorbed in blood and quickly excreted in urine. Brownlee and Tonpin (1941) reported favourably about its bacteriostatic activity against intestinal pathogens. The range used included strains of Sonne, Flexner, Newcastle, Shiga, Coli, Typhoid and Cholera. Sonne strains are found to be most resistant to Sulphanilylguanidine and Shiga and Typhoid strains somewhat resistant. While Sulphanilylbenzamide has proved highly potent in all types.

4. Pharmacological action of sulphonamides

(1) General action and toxicity

The pharmacological action of sulphanilamide has been described in detail as a typical example of the sulphonamide group of drugs.

Prontosil I is said to be pharmacologically indifferent and 10 mgm per kilo cause no change in blood pressure or heart function in cats and rabbits under experimental conditions. No thrombus formation or other deleterious effects were produced by administration of doses as high as 1.0 gm per kilo by the subcutaneous or intravenous route. Smooth muscle from the uterus and large intestine either isolated or in situ was not affected by this compound.

Sulphanilamide hydrochloride is a relatively inert substance. In small dose it first stimulates and then depresses the central nervous system, the depression being manifested by motor paralysis and partial narcosis.

Small intravenous doses cause a strong stimulation of the respiratory centre, whereas large doses depress it; but these effects may have been due to the acidity of the solution of the compound. Death is caused either by paralysis of the respiratory centre or by cardiac standstill since the drug depresses the heart muscle directly. In cat dilatation of the heart, diminution in the amplitude of cardiac contractions, arrhythmia and finally cardiac standstill in diastole are produced. The isolated rabbit's heart also responds to the drug with a diastolic standstill .

Large intravenous doses produce in animals a fall in blood pressure mainly due to depressant effect on the heart and partly also to dilation of the vessels of the splanchnic area. Smooth muscle is depressed by the drug while skeletal muscle such as isolated gastrocnemius of frog is contracted in very dilute solution. In more concentrated solution the contracture disappeared as well as the ability to react to electrical stimuli.

As in the case of other aniline derivatives, these drugs produce a fall of body temperature in rats, probably due to increased dissipation of heat from the skin. There is also a corresponding depression in the gaseous metabolism and a reduction of the respiratory quotient.

Briefly, small doses of sulphanilamide hydrochloride first stimulate and then depress the central nervous system, the depression being manifested by motor paralysis and partial narcosis. Relatively small intravenous doses produce stimulation of the respiratory centre, whereas large doses produce depression and finally paralysis. The heart is depressed and finally stops in diastole.

Toxic effects in animals.—Although in man toxic effects are produced even by therapeutic doses of sulphanilamide, its acute toxicity in mammals is low. The single oral dose of prontosil tolerated by mice was found to be approximately 0.5 gm per kilo and the subcutaneous dose was one quarter of that amount.. Mice tolerated 2.0 gm per kilo of prontosil II orally or intravenously and 4.0 gm per kilo subcutaneously. The toxicity of sulphanilamide was found to be of the same order, since mice tolerated approximately 2.0 gm per kilo either orally or subcutaneously, rabbits 1.0 gm per kilo orally, rats 0.2 to 0.4 per kilo intravenously. P. benzylaminobenzene sulphonamide was less toxic; disulphanilamide, certain anilides, sulphones, sulphides, disulphide, have all been tested and have more or less the same toxicity.

(2) Absorption, distribution, fate and excretion.

Sulphanilamide

Both in animal and human subjects, sulphanilamide when given by the mouth is quickly absorbed and the process is complete in 1 to 3 hours. Absorption is quicker if given in solution than in capsules or tablets. Alkalies hasten absorption. After a single dose the peak in blood concentration is reached in 3 hours; thereafter the concentration falls gradually to zero during the following 24 hours. With repeated administration at intervals of 4-6 hours, a blood concentration of 2 to 10 mgm per 100 ccm can be obtained and maintained. The optimum effective blood concentration is 10 mgm per 100 ccm and this is obtained by a dosage scale of 1 gm per day to every 20 lbs of body weight (Long and Bliss), that is about 7 gm for an average individual of 10 stone weight. Children require 50 per cent more than this and infants three times as much (Banks). Subcutaneous injections do not give a higher concentration in the blood than obtained by oral administration.

Sulphonamides except sulphaguanidine, succinyl sulphathiazole and phthalyl sulphathiazole, are rapidly absorbed from the small intestine and large intestines when they are taken by the mouth; absorption from the large intestine can occur but at a slower rate; as a rule all the drug taken is absorbed before the ileo-caecal valve is reached. Even in the presence of diarrhoea, faecal excretion of such compounds as sulphanilamide is negligible. In conditions of shock also absorption takes place. Sulphapyridine is absorbed somewhat slowly, less regularly and less completely than some of the other compounds; 30 to 60 per cent of sulphaguanidine administered is usually absorbed (depending on the dose), while the rest is excreted in the faeces in high concentration. Little or none of succinylsulphathiazol is taken up from the gut, only 5 per cent of the dose is recovered from the urine.

Distribution.—After absorption into the circulation, sulphanilamide is distributed approximately equally throughout the whole body. It penetrates readily into the cerebrospinal fluid, peritoneal and other effusions. It is not concentrated in any particular organ or tissue, though sulphapyridine accumulates in the liver to double the concentration than that in the blood. "The distribution between plasma and corpuscles varies with the different compounds; if the concentration in the plasma is 1.0, that in the corpuscles is sulphanilamide 1, sulphapyridine 0.6, sulphathiazole and sulphadiazine about 0.3. Similarly, as regards the penetration into the cerebrospinal fluid, if the concentration in the plasma is 1.0, that in the cerebrospinal fluid is: sulphanilamide 1.0, sulphapyridine 0.7, sulphathiazole 0.15-0.4, and sulphadiazine 0.5-0.8. It is possible that these differences may be due to part of the sulphonamide being 'bound' to the plasma proteins; with sulphanilamide only 20 per cent is said to be thus 'bound', with sulphathiazole, about 75 per cent".

Occurrence in blood, tissues, etc. On entering the blood, sulphanilamide quickly penetrates all the fluids and tissues of the body and it appears to be distributed like alcohol or salicylates or urea. The distribution is more or less uniform though the skin, brain, bone and fat contain relatively less. The concentration in plasma is slightly less than in whole blood. The drug seems to be selectively absorbed on to the corpuscles of the blood for the concentration here is 50 per cent greater than in the plasma (Hausen, 1939). In addition to normal body fluids, aqueous and vitreous humors and lens, it is also found in pathological exudates. In normal secretions of the cervix uteri, it is probably present in insignificant amounts. As might be expected, sulphanilamide is found in menstrual blood. It passes

readily into fœtal blood and amniotic fluid. It has been found in the human breast milk 24 hours after oral administration, in concentrations of 0.55 to 2.17 mgm per 100 ccm (Adair, 1938). In milk it is excreted partly unchanged and partly in the acetylated form while excretion continues for sometime after the blood level has become negligible. In the cerebrospinal fluid its concentration is about 75 per cent of that in the blood and the drug appears to pass readily into the cerebrospinal fluid of the patients suffering from meningitis as that of healthy subjects.

In the blood, part of the sulphanilamide is conjugated with other groups especially the acetyl group. Thus sulphanilamide is partly converted into N_4-acetyl-sulphanilamide which can be distinguished from N_2-acetyl-sulphanilamide or sulphacetamide which is therapeutically active. The N_4-acetyl derivatives are probably more toxic than the original compound, acetylation probably taking place in the liver. N_4-acetyl derivatives are therpeutically inactive and they do not give the Bratton and Marshall test, but they can be reconverted into the original form by boiling with acid and alkali. In chemical reports on blood concentration of sulphonamides the figure for the 'free' compound refers to unchanged sulphonamide and the total refers to unchanged plus conjugated compound..

The point of practical importance is that many of the acetyl derivatives are less soluble than original compounds and consequently during excretion through the kidney they tend to crystallise and block the urine passages. The extent to which acetylation occurs varies with different compounds and with the time from commencement of treatment. Sulphanilamide and sulphapyridine are more readily acetylated than sulphathiazole; 20 per cent of the compound in the blood and 25 to 60 per cent of that in urine is in the conjugated form.

It has recently been shown that, though the alkali reserve falls after administration of sulphanilamide and bicarbonate is excreted in the urine, the pH of the blood nevertheless remains constant or even rises.

Concentration in blood

Concentration in blood and excretion. Sulphanilamide taken by mouth is absorbed into the circulation, the blood concentration rising rapidly, being maximal in from three to four hours. "A dose of 2 gm to an adult of average weight usually produces a maximum blood concentration of approximately 2 mgm per 100 cc, one of 4 gm produces a concentration of about 4 mgm per 100 cc and so on. The blood concentration soon falls again, owing to rapid excretion and most of the dose appears in the urine in about 24 hours. Because of this rapid excretion, it is necessary to give the drug in frequent regular doses, *e.g.*, every 4 hours in order constantly to maintain an adequate level of concentration in the blood for successful therapy". .The excretion of the other compounds such as sulphapyridine and sulphathiazole proceeds somewhat more slowly; it is slowest in case of sulphadiazine which takes more than three days to eliminate, only 30-80 per cent occuring in urine.

Briefly sulphanilamide is excreted by the kidneys both in free and conjugated forms, chiefly by glomerular filtration. The rate or elimination depends on the urine flow and not on the plasma level and hence it is possible to wash out the drug by promoting diuresis. Of the drug in the glomerular filtrate, 70 to 80 per cent is reabsorbed by the tubules. Diuresis will thus favour excretion of the drug and a state of impaired renal function will have the opposite effect. After a single dose, the excretion is complete after three days. After the same period with repeated doses a state of equilibrium is obtained between the amount of drug taken and the amount eliminated. To obtain an adequate concentration in the blood it is necessary to use the drug at first in larger doses and then smaller doses repeated at frequent intervals.

"Sulphonamides are excreted in the saliva, in the sweat, and also in the milk, in concentrations similar to those which occur in the blood; they pass through the placenta into the fætal circulation. The amount occurring in milk is too small to exert any effect, toxic or therapeutic, upon a suckling infant unless it is extremely susceptible, and sulphonamides need not usually be withheld from pregnant or lactating women on account of the child. Nevertheless, it is probably wise to exercise reasonable caution on such occasions".

The action of other sulphonamide compounds is, in the main, the same as sulphanilamide. There is only question of degree. Sulphathiazole is more quickly absorbed (in 3 hours) and sulphadiazines are less readily absorbed (in 6 hours). The repetition of doses has to be arranged accordingly. Sulphathiazole is very readily excreted in the urine and it is sometimes difficult to maintain proper concentration in the blood. The diazines are less rapidly excreted. Crystalluria is common with sulphathiazole and diazines. All compounds readily penetrate the tissue and occur in body fluids and excretions.

(3) Action on micro-organism

(a) *In vitro action against bacteria.*—Prontosil was found by Domagk to be ineffective against bacteria *in vitro* even in high concentrations, but was active *in vivo* against the same organisms. Later work showed that in concentration of 1 in 10,000 sulphanilamide has bacteriostatic action against hæmolytic streptococci, pneumococci, *Cl. welchii* and *Brucella melitensis.* In concentrations higher than 1 in 1,000 it has a bactericidal action also against some other organisms. When the culture media employed are less favourable for growth, higher concentrations of sulphanilamide have a bacteriostatic or even a bactericidal effect on staphylococci and a number of gram negative organisms. It is for this reason that sulphanilamide is useful in urinary infections.

Sulphapyridine inhibits the growth in vitro of large inocula of pneumococci in leucocyte-free human blood in concentration of 1 in 250,000 to 1 in 8,000. When leucocytes were present concentrations of 1 in 32,000 killed the organism. It is, therefore, more effective than sulphanilamide. It is also more effective against hæmolytic streptococci, *E. coli* and *E. typhi.*

Sulphathiazole has marked bacteriostatic action against streptococci as well as staphylococci. It also acts on *Str. faecalis* which is not acted on either by sulphanilamide or sulphapyridine. Sulphathiazole and thiazole derivatives have the most powerful bacteriostatic action of all sulphonamide drugs on colon-typhoid-dysentery group. Growth of both hæmolytic and non-hæmolytic enterococci is inhibited.

(b) *In vivo action.*—A large volume of work was carried out with sulphanilamide and other compounds on experimental animals. It was shown that sulphanilamide was effective against group A of hæmolytic streptococci which cause a large number of human infections. Infections in mice with Group B are resistant. Group C are moderately susceptible. Infections with staphylococci and gonococci are less susceptible; pneumococcal and meningococcal infections were not susceptible; *B. typhosus* and Paratyphoid B infections in mice were occasionally cured and those with *Cl. welchii* were beneficially affected. Sulphanilamide modified the course of brucella infection in guinea pigs favourably. Experimental syphilis in rabbits, virus infections, such as experimental poliomyelitis in monkeys, dog distemper were not touched; lymphopathia venerium infections in mice and guinea pigs were beneficially affected.

Sulphapyridine was more effective in vivo in pneumococcal, staphylococcal and meningococcal infections in mice than sulphanilamide. Sulphathiazole is quite effective against pneumococcal, staphylococcal, streptococcal and meningococcal infections.

5. Mode of Action of Sulphonamides

Bactericidal and bacteriostatic action.

(1) *Direct or indirect action.*—When dealing with the mechanism of the action of any chemotherapeutic agent it is of prime importance to learn on the one hand, as to what extent the drug is capable of direct action on the micro-organisms, and on the other, the extent to which this action is influenced or mediated in the tissues of the host. Of great importance also is the comparison between the dosages that are effective in the test tube and those in the body. If for instance large amounts of the drug are required to kill the organisms in vitro and only infinitesimal fractions of the drug are effective in vivo in the animal, it becomes obvious that the compound has either undergone a chemical change in the body or that unknown in-vivo factors have enhanced its action. This is exactly what was found in the experiments with prontosil. In the test tube prontosil was practically inert but in experimental tests in mice, it was found to exercise a powerful action which quickly freed the tissues of *Str. haemolyticus* and effected a cure. Prontosil is believed to be reduced in the body to sulphanilamide which is said to be the effective agent. The

chemotherapeutic property of prontosil could not have been discovered except by trial in infected subjects, but in the case of sulphanilamide, bacteriostatic action was observed by in vitro tests when using a small number of micro-organisms for the inoculum. Thus Colebrook and his colleagues (1936) obtained bacteriostatic action with sulphanilamide in human blood and in human serum. In human blood 15,000 streptococci added to 1 ccm of blood containing sulphanilamide in a concentration of 1 in 12,000 were destroyed partially in six hours and completely in 24 hours. Similar effects were obtained in the blood with its leucocytes removed. This bacteriostatic effect was much greater than the bactericidal effect of normal human blood. The same observers stated that the bacteriostatic power of the blood of rabbits, mice and monkeys could be increased by feeding with sulphanilamide. For example after the oral administration of 4 gm of sulphanilamide to a monkey, the blood was shown 3½ hours after its administration to be 1,000 times as bacteriostatic as normal monkey blood; after 24 hours it was only 100 times and after 48 hours the power was only slightly above that of the normal animal. Similar results were obtained in man by the same observers. The blood of one normal person collected 2 hours after being given 1.5 gm of sulphanilamide orally was shown to cause complete destruction of 7,200 streptococci in 100 ccm of blood, whereas before treatment the same person's blood could not prevent 28 streptococci from multiplying to a very large number in 24 hours.

Prevention of multiplication of organisms. The sulphonamides act upon susceptible micro-organisms by their bacteriostatic rather than bactericidal action, that is to say their multiplication is prevented. If the multiplication of micro-organisms can be held in check for sometime the defence mechanisms of the body can usually overcome the infection by destroying the invading bacteria. The clinical response to sulphonamides is rapid when the defence mechanisms are efficient and the infecting bacteria are sensitive to the drugs. Direct bactericidal action is probably not produced by concentration of sulphanilamide reached in the tissues with doses given clinically, but recovery may occur without complete eradication of the infecting organism from an original focus and this probably accounts for relapses.

It should be clearly understood that sulphonamides do not neutralise bacterial toxins to any great extent, though there is some evidence that they may inactivate certain endotoxins.

Leucocytes have been shown to be essential for bactericidal action of these drugs. It has been shown that the presence of leucocytes in the culture medium allows otherwise bacteriostatic concentrations of the drug to kill the organisms. In vivo it has been shown that active phagocytosis occurs in recovery from infection with sulphanilamide therapy. In mice with granulopoenia, even intensive sulphanilamide therapy does not cure infection with haemolytic streptococcus. Phagocytic activity is, therefore, an essential part of the sterilising effects of sulphanilamide therapy in infections in animals. The action of the drug appears to be primarily on the bacteria which are altered in such a way as to be easily ingested by phagocytosis. The drug does not in any way stimulate the mobilisation of the macrophages.

Sulphanilamide does not enhance the bactericidal value of human serum nor does it promote antibody formation. It does not probably hinder it either. The treated animals show no immunity upon re-infection.

Changes in bacteria.—Morphological changes are produced in mircro-organisms when acted upon by sulphanilamide; streptococci may show increased length of chains, swelling and alteration of staining reactions. Meningococci swell up, become irregular and pyriform in shape and show staining abnormalities. The changes, however, do not appear to alter their virulence when injected into animals. Such morphological changes suggest that sulphanilamide depresses the

metabolic activity in the body of the bacteria which is essential for invasion of animal tissues. It is suggested that sulphanilamide is converted into a substance which combines with the proteins available for bacterial nutrition, rendering it unfit for assimilation.

(2) *Role of para-aminobenzoic acid.*—The action of sulpnonamides can be neutralised by small concentrations of p-aminobenzoic acid,



or less effectively by certain other compounds such as methionine or even peptones. It would appear that p-aminobenzoic acid is essential for the growth of bacteria and while most of the bacteria can synthesize this compound, for some others it must be supplied from outside and is therefore a growth factor. The sulphonamides are sufficiently similar in chemical composition to p-amino-benzoic acid, to be used instead by the bacterial organisms if present in greater concentration. They do not, however, serve the metabolic functions equally well, and the result is interference with bacterial multiplication.

The enzyme-system has a greater affinity for p-aminobenzoic acid than for sulphonamides and, therefore, to be effective, the sulphonamides must be present in much greater concentration. It has been calculated that to counteract "in vitro one part of p-aminobenzoic acid, a concentration of 1,600 parts of sulphanilamide, 100 parts of sulphapyridine, 36 parts of sulphaguanidine is required". Pus and necrotic tissue contain substances antagonistic to the antibacterial action of sulphonamides, and, therefore, these drugs exert less effect than usual if they are applied locally to wounds containing pus or sloughs. Substances having an inhibitory action have also been demonstrated in fresh muscle, spleen and pancreas, and in pleural, pericardial and synovial fluids; such substances may interfere with the therapeutic action of the drugs in vivo.

The action of sulphonamides can also be neutralised, besides p-aminobenzoic acid, by other chemical compounds containing the p-aminobenzoic acid group, although less rapidly. Examples of these compounds are procaine (the diethylamino-ethyl ester of p-aminobenzoic acid, also known as novocain, planocaine, etc.) larocaine, tutocain, butyn, benzocaine (anaesthesin) and orthocaine (orthoform). If these particular local anæsthetic drugs are used at the same time as sulphonamide drugs the effect will be weakened. It should be borne in mind that these anaesthetics also give the same colour reaction as the sulphona-mides in Bratton and Marshall's test; they should not, therefore, be employed in obtaining samples of cerebrospinal or other fluids for estimations of the drugs.

Such local anaesthetics which do not contain p-aminobenzoic acid group, *e.g.*, cocaine, tropacocaine, benzemine (eucaine), amylocaine (stovaine), nupercaine, phenacaine, amethocaine (decicaine) and metycaine can be used.

Chemical structure and bacteriostatic activity. It has been found that shifting of amino group of the sulphonamide compounds from the para to the ortho or meta position, removes the bacteriostatic activity of these compounds. Substitution of the p-amino radical with other groups, produces compounds which have no bacteriostatic effects, or which are not neutralised by p-aminobenzoic acid, as for example is the case with marfanil (homosulphanilamide). The addition of other groups to the p-amino radical abolishes the activity as in the case of acetylation, which occurs in the liver in producing the conjugated forms. If, however, the added groups can be separated off in the body, as for example in succinyl-sulphathiazole and phthalyl sulphathiazole, the bacteriostatic activity remains intact. Addition of other substituted groups to the amido group may increase the bacteriostatic activity of sulphanilamide. This increased activity varies with the type of group used for substitution. Thus the increase is small if this group is acyclic as in sulphacetamide and sulphaguanidine, greater if an isocyclic group is used, and maximum with the addition of heterocyclic groups, as in sulphapyridine, sulphadiazine and sulphapyrazine, especially if this group contains different hetero-atoms as in sulphathiazole.

(3) *Ionic dissociation.*—The sulphonamides undergo ionic dissociation in solution and behave to a certain extent like weak acids, their activity probably depending not upon the whole molecule, but only upon the anion. "Within certain limits the bacteriostatic activity tends to be greater in alkaline solutions, since more ions are available under these conditions. Sulphonamides which are about 50 per cent dissociated at the reaction of the body fluids, *e.g.*, sulphathiazole, tend to be more active than those in which dissociation is about 3,000 times less, *e.g.*, sulphanilamide. Although for this and other reasons the action of some sulphonamides is quantitatively greater than that of others, there is little evidence for a qualitative difference apart from the exceptional case of "Marfanil" whose mode of action is different." The fact that sulphanilamide is effective against streptococci, sulphapyridine against streptococci and pneumococci and sulphathiazole and sulphadiazine against streptococci, pneumococci and staphylococci is according to many authorities due not to the action of any particular drug being specific for certain organisms, but rather to the less susceptible organisms such as pneumococci and staphylococci responding only to the more active compounds. The differing rates of absorption and excretion do render the use of certain compounds advantageous for certain types of conditions, thus sulphanilamide, on account of its ready solubility is useful for local application to wounds, though the less soluble sulphathiazole has greater bacteriostatic power. Sulphadiazine produces particularly high blood concentrations because of its slow excretion, while sulphaguanidine and phthalylsulphathiazole are effective against organisms in the lumen of the lower part of the alimentary canal because they are either very slowly or incompletely absorbed and for that reason reach this region in high concentrations. The differences in the fields of usefulness of the various compounds probably depend much more upon differences in their physical and pharmacological properties than upon any specificity between drugs and bacteria.

(4) *Drug resistance.*—When micro-organisms are exposed in vivo or in vitro to concentrations of sulphonamides which are not potent enough to destroy them all, the survivors often become resistant to the further action of these drugs. The organism may eventually become capable of growing in comparatively high concentrations of the drugs under suitable conditions. This acquired resistance of bacteria to sulphonamides is analogous to the well-known arsenic-resistance which may develop in trypanosomes, and also like trypanosomes, once it is established, it persists through many generations of the bacteria. This phenomenon has been shown to occur in case of pneumococci, gonococci, streptococci, B. coli and dysentery bacilli, and probably also in other bacteria, but its frequency in clinical practice has not been established. Cases have been described of pneumococcal infections which underwent prolonged treatment with sulphonamides, and which eventually became completely resistant, but when short intensive courses are given the development of drug-resistance is probably rare. In any case care should be taken to avoid fostering the development of drug resistance in the infected by avoiding prolonged treatment with small doses of sulphonamides. Once organisms have become resistant to any sulphonamide compound, they become correspondingly resistant to all the other sulphonamide compounds (except Marfanil), but it may be found that a more active sulphonamide, *e.g.*, sulphathiazole, will still exert some antibacterial action when less active compounds, *e.g.*, sulphanilamide have proved ineffective. Bacteria which have become resistant to sulphonamides still retain their original sensitivity to compounds of other types, *e.g.*, penicillin, gramicidin, the acridine antiseptics, etc.

(5) *Estimation of potency of sulphonamides.*—Estimations of potency in vitro are determined by measuring the minimal concentration required to inhibit the growth of a susceptible organism. Using a standard inoculum of *B. coli,* it was found that while the necessary concentration varied when different media were employed the relative proportion for different drugs was always the same. These proportions were also found to correspond to the *in vivo* activity, based on figures obtained from effective blood concentrations and percentage survivals. It has also been shown that the bacteriostatic potency of a sulphonamide compound is in proportion to its ability to counteract the antibacteriostatic action of p-aminobenzoic acid. Wood (1942) found that the relation of the minimum amount of p-aminobenzoic acid required to prevent bacteriostasis, to the concentration of sulphonamide used, was constant, despite wide variations in the latter. He used the term *bacteriostatic constant* which equals: molar concentration of p-aminobenzoic acid/molar concentration of sulphonamide, at which bacteriostasis is just prevented. He employed an inoculum of 10,000 viable *B. coli* in plain synthetic broth at 37°C, and took readings at the end of 5 days. An alternative and more satisfactory method of expressing the potency is the *sulphanilamide coefficient*:-the bacteriostatic constant of sulphanilamide/the bacteriostatic constant of the sulphonamide compound under test, or the minimum bacteriostatic concentration of sulphanilamide/the minimum bacteriostatic concentration of the sulphonamide under test.

The average Sulphanilamide Coefficient adjusted to constant range taking figures from a number of workers, who used *B. coli, Staph. aureus,* pneumococcus type I and III, is given below:—

Sulphanilamide	1
Succinyl-sulphathiazole	0.6
Sulphaguanidine	4
Sulphacetamide	8
Sulphapyridine	13
Sulphamezathine	13
Sulphamerazine	20
Sulphadiazine	36
Sulphapyrazine	50
Sulphathiazole	50

The consensus of opinion at present is that sulphadiazine and its homologues, sulphamethazine (sulphamezathine) and sulphamerazine are the most effective and the least toxic of these compounds. On account of their comparatively slow excretion the interval between doses can be increased to 6 to 8 hours which is a great advantage from the point of view of the patient. Of the sulphonamides used for intestinal conditions, sulphaguanidine is being replaced by sulphasuccidine or sulphathalidine. A soluble compound with nearly neutral reaction for parenteral injection is yet to be discovered.

6. Routes of administration

(1) Oral route

The sulphonamide drugs are usually given by the oral route. In order to facilitate absorption, the tablets must be crushed and taken with half to one ounce of a mixture containing sodium bicarbonate and sodium citrate, 30 grains each to the fluid ounce. After taking the drug the mouth should be thoroughly rinsed with water in order to dislodge the powder from the crevices

of the mouth. If the patient is in a state of coma, the drugs are given by nasal catheter or by the stomach tube, the powder being sufficiently diluted to prevent the sediment from blocking the tube.

The oral route is the method of choice because of its simplicity and practicability and it should always be used unless it is contra-indicated by the condition of the patient. It has been shown that approximately the same concentration of sulphanilamide was obtained in 3 to 5 hours after administration of the same amount of drug by either the oral or the subcutaneous route.

Rectal or subcutaneous administration is used as a substitute for oral administration when this method cannot be used as for instance after operation in the region of the stomach when there is no need to raise the blood concentration readily.

(2) Parenteral route

(a) *Subcutaneous administration.*—The subcutaneous route is useful in cases of persistent nausea and vomiting where hypodermoclysis is indicated. It is also useful in rapidly building up the desired concentration of the drug in the blood when the oral route has failed to do so.

(b) *Intramuscular administration* is used as a substitute for intravenous administration when good veins are not available or for some other reasons intravenous injection is not desirable. This method is less satisfactory and certain amount of risk is involved with the sodium salts of sulphapyridine, sulphathiazole and sulphadiazine on account of the high alkalinity of the solution. It has no particular advantage over the subcutaneous route.

(c) *Intravenous administration.*—This route has no justification in routine treatment, as absorption is sufficiently rapid by the oral and subcutaneous routes. It should be used when it is desired to attain high concentrations of sulphonamides in the blood. This is usually done at the beginning of the treatment of a severe infection or when for certain reasons oral administration is not possible.

(3) Strength of solutions

Solutions of sodium salts of the sulphonamides may be used for intravenous or intramuscular injections except in the case of sulphanilamide where no such salt is available. The sodium salts of sulphathiazole or sulphapyridine are given in 10 per cent solution and for sodium-sulphadiazine 5 per cent solutions are recommended. As these solutions are higly alkaline they are liable to set up irritation and necrosis when they escape into the tissues. They can, however, be given intravenously and if they are given very slowly and no material is allowed to escape outside the vein, no deleterious effects are produced. Intramuscular injections are liable to produce considerable damage and if it is necessary to use this route, the number of injections should be as few as possible and the distance between the site of each injection should not be less than three inches. The injections should be given deep into the muscles and as far away as possible from the important nerves; vastus externus muscle is usually selected for this purpose as it has no important structure underneath. If injections are given in the neighbourhood of a large nerve such as the sciatic, serious damage may occur. Solutions of sodium salts should under no circumstances be given intrathecally.

Sodium salts of sulphapyridine and sulphathiazole are supplied in powder form, in ampoules containing 1.0 gm each. Both for intravenous and intramuscular injection the contents of an ampoule are dissolved in 10 ccm of sterile distilled water immediately before use. Sodium sulphadiazine is usually supplied in bottles containing 5.0 gm each and a 5 per cent solution can be freshly prepared. It is rarely necessary to give more than one intravenous injection but in cases of persistent coma or vomiting, injections must be continued. 3.5 gm are given every 12 hours for an average adult of 60 kgm (9½ stone) weight. Sodium salts have also been given by continuous drip infusion in saline.

A saturated solution of sulphanilamide (0.8 per cent) is prepared by dissolving 1.0 gm of the crystalline sulphanilamide in 125 ccm of sterile physiological saline solution. This solution can be given by the intravenous route or by continuous drip method. The soluble form of sulphanilamide known as sulphanilamide L.S.F. which is issued in 15 per cent solution in ampoules may be used for parenteral injections.

By adding excess of sulphanilamide to warm water (26 gm to a Winchester bottle of 2,250 ccm capacity) and allowing it to cool at room temperature, a saturated solution is prepared. The supernatant fluid is decanted off and will contain about 0.8 per cent of sulphanilamide. The solution containing the required quantity of this drug is diluted to 300 ccm (10 fl oz) with one per cent glucose in physiologic saline or physiologic saline alone. Per rectum the drug is given every four hours in slightly larger doses than used for oral administration. The concentrations of the drug obtained in the blood by this method are lower than those following oral administration and there is longer time lag. Sulphapyridine should not be given by the rectal route. Sodium salts of sulphapyridine, sulphadiazine or sulphathiazole are given by the subcutaneous route, when administration by the mouth is not possible, 5.0 gm of the salts being given by continuous drip method (hypodermoclysis). The pH values of the solutions are 10 and 9 respectively, but inspite of this they usually cause no serious reactions. The concentrations of the drug in the blood when given by this route show large variations.

7. Dosage of sulphonamide drugs.

General scheme of dosage.—The effective dosage in the case of important sulphonamide drugs in use has been given under individual drugs at the beginning of this section. Medical Research Council War Memorandum No. 10 (1945) has laid down a scheme of dosage for curative and prophylactic purposes as result of experience gained both in Civil and Military practice. This authoritative scheme is reproduced below:—

The dosage given is suitable for subjects of average weight who are given sulphanilamide, sulphathiazole, sulphadiazine, sulphadimethyl pyrimidine or sulphapyridine if tolerated; somewhat smaller doses are required in case of sulphamerazine. The weight of the average adult may be taken as 60 kgm (9½ stone); if there is wide departure from this, corresponding allowances in dosage should be made. Children, weight for weight, have a better tolerance than adults for sulphonamide drugs. In infants, tolerance per unit of weight has been estimated as three times that of adults. The monograph states.—"This table is intended for general guidance, and in particular instances the doses may often have to be modified to suit the individual patient or the special properties of the drug given; the manufacturers' own recommendations regarding dosage will be of help here, and should be carefully considered, especially in case of the newer sulphonamides."

DOSAGE OF SULPHONAMIDES

Severe Infection (endangering life)

	Adults	Children		
		0-3	4-10	11-15
Initial dose	2-4 gm intravenously 1.5 gm by mouth	0.5 gm intravenously 0.5 gm by mouth	1 gm intravenously 0.75 gm by mouth	1-2 gm intravenously 1 gm by mouth
Followed by:— 1st period, 2-3 days	1.5 gm 4-hourly	0.5 gm 4-hourly	0.75 gm 6-hourly	1 gm 4-hourly
2nd period, 2 days (approximately two-third of dose of 1st period)	1 gm 4-hourly	0.5 gm 6-hourly	0.75 gm 6-hourly	1 gm 6-hourly
3rd period, 2 days (approximately one-third of dose of 1st period)	1 gm 6-hourly	0.25 gm 6-hourly	0.5 gm 6-hourly	0.5 gm 6-hourly

Milder or "Moderate" Infections

	Adults	Children		
		0-3	4-10	11-15
Initial dose	2 gm	0.5 gm	0.75 gm	1 gm
Followed by:— 1st period, 2 days (unless otherwise stated)	1 gm 4-hourly	0.5 gm 4-hourly for 12-24 hours	0.5 gm 4-hourly	0.75 gm 4-hourly
2nd period, 2 days (unless otherwise stated)	1 gm 6-hourly	0.5 gm 4-hourly for 3-4 days	0.5 gm 6-hourly	0.75 gm 6-hourly
3rd period, 2 days	1 gm 8-hourly	0.25 gm 6-hourly	0.25 gm 6-hourly	0.5 gm 6-hourly

The duration of a course is normally 6-7 days, and it should rarely be prolonged beyond the seventh day."

Prophylactic dosage

"This will vary according to circumstances, but some examples may be given:—

"(*a*) For children undergoing tonsillectomy, when the presence of a streptococcal infection is suspected or known.—

Drug: Sulphanilamide	Age 4-6	Age 7-15
Pre-operative dose, given 2 hours before the operation	1 gm	2 gm
Post-operative doses, starting 4 hours after the operation and continuing at four-hourly intervals for 2 days	0.25 gm	0.5 gm

"(*b*) For women in labour, when there is known risk of infection by hæmolytic streptococci.—

Drug: Sulphanilamide

First dose, at beginning of labour	2 gm
First day, at 4-hourly intervals, beginning 4-hours after the first dose	0.5 gm
Second and third days, at 6-hourly intervals	0.5 gm

"(*c*) To prevent urinary tract infection, *e.g.*, when an indwelling catheter is required for some days.—

Dose, 1st day	1 gm	0.5 gm
	4-hourly	4-hourly
Subsequent days —	1 gm	0.5 gm
	6-hourly	6-hourly

Summary of dosage:—

In severe illness. For first 2 or 3 days 9 gm daily, second 2 or 3 days 6 gm daily and third 2 or 3 days 3 gm daily. Total 40 to 50 gm.

In mild illness. For first 2 or 3 days 6 gm daily, second 2 or 3 days, 4 gm daily and third 2 or 3 days, 2 gm daily. Total 24 to 32 gm.

For children of six years half the adult dose; for a child of three years and under, one-third of the adult dose.

An initial loading dose (4-3 gm in adults) should always be given when treatment is begun.

(*d*) There is no evidence that the sulphonamides are effective against the primary infection in influenza, measles and pertussis. The drugs may, however, be used to control the secondary complications—in particular, acute otitis media, bronchitis and broncho-pneumonia—which commonly follow. "As the complicating infections may be due to one or other of a variety of pathogens—hæmolytic streptococcus, pneumococcus, staphylococcus, influenza bacillus—one of the more generally active sulphonamides should be prescribed. Experience favours the giving of the drug at the first signs of secondary fever, rather than as a routine prophylactic in every case. There is some evidence, however, that the incidence of bronchopneumonia and suppurative otitis media following measles may be reduced by routine prophylaxis with sulphathiazole or sulphapyridine given for 5 days from the appearance of the rash (Banks: Practitioner, 1942, 149, 297). This will not prevent infections from being occasionally contracted from other patients after the drug is stopped."

Dose:	0-2 years	2-6 years
	0.25 gm	0.5 gm
	6-hourly	6-hourly

In severe infections best results will be obtained if the concentration of the drug in patients' blood attains a certain level. This should, therefore, be determined during the first two or three days, 6 to 12 hours after beginning of treatment and again at 24 to 36 hours after. It is not always possible to insure the desired concentration from a standard dosage because there are great variations in the absorbability of the drug from the gastro-intestinal tract. Besides this an uncertain amount of the drug absorbed becomes therapeutically inactive by acetylation. Vomiting if it occurs is also another factor. In children often too small doses are given. The estimation of sulphonamide in the blood may be difficult but micromethods have been introduced to make it possible to obtain a rough estimate of their concentration even from a drop of blood from the finger or the lobe of the ear. The importance of these estimations during early stage of exhibition of these drugs is that they will give a warning if the dosage is inadequate without waiting for clinical indications which may be difficult to interpret. If a patient does not respond to treatment for three or four days the estimation of concentration of these drugs in the blood combined with the sensitivity of the organism may explain the cause of the failure and suggest further action. While estimations of sulphonamides in the blood are important in severe infections it is not essential that they should be done in all cases or that the drug should be withheld if laboratory facilities for such examinations are not available.

8. General considerations regarding use of sulphonamides.

The first important point is that in the treatment of acute infections a large initial or loading dose of drug should be given in order to get the blood concentration of the compound to an effective level as carefully as possible. In very severe cases it is desirable to do this by giving the first dose either intravenously or intramuscularly.

The reasons for this are:—

(1) The sulphonamides are much more effective on a smaller number than on a large number of micro-organisms. It is well known that hæmolytic streptococci are able to multiply in human tissue fluids. If however, an effective dose of sulphonamide is given during the rapid growth phase, acute infections are likely to be aborted before any serious damage is done and before the establishment of the suppurative stage which is much less amenable to treatment with sulphonamides

(2) It has been shown that sulphonamides have a more marked antibacterial action at fever temperature than at the normal temperature of the body.

(3) It is also possible that exposure of micro-organisms to gradually increasing dose of sulphonamides, as would occur if the patient is treated with small doses, may produce development of drug resisting strains. The possibility of this contingency is much less if the drugs are given in full therapeutic doses from the very begining.

It should be clearly understood that the greater the risk to life from the infection, the larger the dosage required. Doubling the dose, however, does not double its therapeutic effect, but probably increases it only by a 5th to 3rd. Further higher doses require greater precaution against the deleterious effects of the drug and frequent leucocyte counts to give warning of agranulocytosis have to be done. Sufficiently larger quantities of fluid to insure larger quantities of urinary secretion so as to minimise the risk of blockage of urinary passages must be taken. The fluid intake should not be less than five pints (3,000 ccm) daily in an adult and to this the amounts lost through vomiting, diarrhoea or sweat should be added. It should be appreciated that in India during the hot weather 3 pints or more may be lost by profuse sweating. The criterion to be kept in mind is that under no circumstance should the output of urine be allowed to fall below 50 ounces (1500 ccm) per day.

Medical Research Council War Memorandum No.10 lays down the following detailed directions regarding the amount of fluid to be taken:—

"With all the usual sulphonamides except sulphanilamide, sulphacetamide and probably sulphadimethylpyrimidine, it is essential to give ample fluids so as to secure a good flow of urine and guard against urinary obstruction; it is wiser to make this a general rule for all the drugs; at the same time (except with sulphapyridine), obstruction is less likely to occur if the urine is kept alkaline. The exact amount of fluid and alkali required to be given daily for this purpose will depend upon the circumstances, but as a general rule 0.5 liter (1 pint) of fluid should be given for each gramme of sulphonamide up to a maximum of about 3.5 liters (6 pints) for an adult; the essential principle is to maintain a urinary output of over 1,500 cc (50 fl. oz.) in the 24 hours. Similarly, as regards alkalis, the important thing is to arrange that the urine is alkaline to litmus paper; this can usually be achieved by giving 1.3 gm (20 grains) of sodium bicarbonate and 1.3 gm (20 grains) of sodium citrate with each dose of sulphonamide. For infants and children, smaller doses of alkali should be given."

"The maintenance of a large fluid intake during sulphonamide therapy has the added advantage of assisting solution of the drugs in the upper part of the alimentary tract."

Combined Therapy:—It has been pointed out that it is advantageous to administer two or more compounds conjointly in the treatment of severe infections. This permits usually smaller doses of each compound than ordinarily required of either drug alone, with

possible prevention or reduction of toxic reactions. It is also said that this reduces chances of production of drug resistance in the organism concerned in infection. Experiments on mice show that the compounds probably act synergistically and additively. A mixture of equal parts of sulphathiazole, sulphadiazine and sulphamerazine is said to reduce or eliminate renal obstruction due to crystalluria without administration of alkalis. Allergic reactions (drug rashes, fever, etc) are also less and blood and urine concentrations are higher.

A combination of sulphadiazine and sulphamerazine has been recommended, the initial dose being 0.09 gm per kilo body weight for patients weighing upto 40 kilo. Beyond that the initial dose is 4 gm and maintenance dose 1.0 gm every 8 hours, day and night.

Sulphonamides such as sulphadiazine combined with antibiotics such as penicillin or streptomycin are more effective than either drug singly.

(1) Concentration in blood.

The concentration of the drugs in the blood must be maintained at an effective level throughout the 24 hours of the day, particularly during the acute phase of infection. This means that the drug should be given at 4-hourly intervals whether the patient is asleep or awake. Approximate indications of the concentration of various sulphonamides in the blood which are likely to be attained with the dosage recommended are given in the following table:—

	Severe infections endangering life: 100 ccm mgm per	Milder cases mgm per 100 ccm	Prophylaxis mgm per 100 ccm
Sulphanilamide	7-10	4-6	2-3
Sulphapyridine	7-10	4-6	1-2
Sulphathiazole	6-7	3-5	1-2
Sulphadiazine	10-15	6-10	2-4

These concentrations are likely to be effective in the majority of infections with the sensitive organisms, *e.g.*, pneumococcus, meninogococcus, haemolytic streptococcus, etc. In cases of less sensitive organisms the concentration of sulphonamides in the patient's blood should be higher. It should also be remembered that besides a few micro-organisms developing resistance to sulphonamides, there may be naturally insensitive strains among sensitive organisms. The degree of sensensitiveness of the organisms can be determined in vitro and this test should be performed in cases where there is unsatisfactory response to the drug. Higher concentrations of sulphonamides in the blood will be required in the treatment of a heavy infection than with an incipient one. As the general condition of the patient improves in acute infections, the dose of the drug should be gradually reduced, but it should not go below sub-effective blood level. The administration of drug should not be stopped too soon as there is risk of recurrence; at the same time if the drug is not given for two to three days after the temperature comes down to normal, it gives a clinical evidence of recovery. The total duration of the course is from 6 to 7 days and it should be rarely prolonged beyond the 7th day. It is advisable to do a leucocyte count if the treatment has to be prolonged beyond 6 days or more than 300 gm of the drug have been given (Succinyl or phthalyl sulphathiazole excluded).

If there is no definite improvement within 3 or 4 days inspite of adequate dosage, the concentration of the drug in the blood should be tested and the situation with regard to the sensitiveness of the infecting organisms to the drug should be determined. If the organism is relatively insensitive a more active sulphonamide drug may be given. The action of sulphonamides is also inhibited by peptones produced in an undrained pocket of pus or in necrotic tissue in a wound produced by disintegration of cells and bacteria. This condition should

be dealt with by drainage. The natural defences of the individual may be poor and may not be able to deal with the organism inspite bacteriostasis produced by the drug as occurs in old and infirm people.

In sub-acute and chronic infection it is not necessary to give a large first dose of the drugs. Where prophylactic action is desired smaller doses may be used because there will be fewer microbes to be dealt with in the initial phase of an infection.

In urinary infections smaller doses are desirable than in case of general infections because concentration of these drugs in the urine is much higher than in the blood and tissues. Total dosage of sulphonamides, midnight to midnight, is recorded on the patient's temperature chart each day to insure that the drugs are not continued for too long. The dose should be recorded in terms of grams and not in terms of tablets.

(2) Cautions and contra-indications

It was experimentally demonstrated that incidence of sulph-hæmoglobinaemia was increased if sulphates and particularly magnesium sulphate was used as a purgative. The reason given was that its cathartic effect brought the unabsorbed food into the large intestine, where it fermented and liberated sulphides. Sulphæmoglobin, however, is not the common cause of cyanosis and these agents do not increase its incidence. Niblack (1941) showed that diets including eggs and onions and administration of various sulphates did not appear to have increased the frequency of cyanosis.

Pregnancy if present is not a contra-indication to the use of sulphonamide drugs, although they pass through the placenta and attain the same concentration in the foetal blood as in the maternal blood. Prolonged use of these drugs, however, is not recommended.

Sulphonamide drugs are contra-indicated where severe toxic reactions have followed their previous use. They should be given with caution in cirrhosis of the liver as they give rise to toxic effects more frequently. In infectious hepatitis due to susceptible bacteria they are beneficial. If anæmia, granulopœnia or jaundice are not due to sulphonamides the drugs are not contra-indicated.

9. Toxic effects of sulphonamides.

(1) Introductory

Sulphonamides are very potent drugs and are liable to produce toxic reactions. It is necessary, therefore, to take every precaution when these drugs are being administered.

Many of the toxic effects described below do not commonly occur. With the exception of crystalluria, these are either due to hypersensitivity to the drugs or to their toxicity. Serious effects have been produced in the hypersensitive even by transfusion of blood from a donor receiving prophylactic doses of sulphonamides. Side-effects may not appear for 10 to 14 days. Reactions occur earlier in those who have received previous doses of the drug.

Skin tests for general sensitivity and patch test for skin sensitivity have been devised.

Previous use of drugs.—Before beginning treatment with sulphonamide drugs it should be ascertained wherever possible whether the patient had these drugs on any previous occasion and if so whether he suffered from any toxic effects. If a drug of this group has been previously taken the amount consumed by the

patient should be taken into account in planning future dosage. Patients who have had toxic reactions previously may even get more severe reactions if the drugs are taken again. Patients who are hyper-sensitive to one sulphonamide compound may not necessarily be hyper-sensitive to another such compound. In such individuals, where clinical condition permits delay, a small test dose of the drug (0.1 to 0.3 gm) should be given 12 hours before the main treatment is started. The patient is carefully watched and if toxic effects appear the drug should be immediately discontinued. Cases have been recorded in which first course of sulphonamide treatment produced no ill effects but a second course given weeks or months later produced rigors, rise in temperature and prostration. This is probably due to the patient becoming sensitized to the drug and this is more common with sulphathiazole than with other drugs. Such a possibility should always be kept in mind although hyper-sensitivity is not a very common occurrence. Hypersensitive patients can be desensitized by giving small initial doses of the drug for 24 hours. General hypersensitivity to sulphathiazole has followed after local application of this drug to skin lesions.

Toxic symptoms and signs.—Patients who are under sulphonamide treatment should be seen by the medical man in charge of the case at least once a day and the early symptoms of toxic reactions, *e.g.*, headache, nausea, vomiting or malaise. should be carefully looked for.

Cyanosis frequently occurs but it is usually of little serious significance. The presence of any rash, jaundiced sclerae, paleness of mucous membranes (due to acute hæmolytic anæmia) may be observed. The temperature of the patients should be carefully recorded. Such symptoms as rise of temperature, lassitude, headache and sore throat especially if they occur after the 8th day, suggest acute agranulocytosis; in such cases the drug should be stopped immediately, and differential leucocyte counts should be done. If after a period of normal temperature, fever recurs in the course of sulphonamide therapy, the drug should be stopped, or if recently discontinued, should not be resumed unless it is made certain that the fever is due to recurrence of the infection under treatment. The blood should be examined for possible granulocytopœnia. In cases in which sulphonamide therapy has to be stopped because of toxic reactions, large quantities of fluids (5,000 ccm or 9 pints daily) should be given to accelerate excretion of the drug. It is advisable not to give fluid by the intravenous route as it is liable to produce œdema of the lungs.

Pain in the kidney region and hæmaturia with decreased urine secretions are indications for immediate stoppage of the drugs. The risk of kidney affection is particularly great when sodium salts of these drugs are given intravenously and the blood thus becomes flooded with excess of these compounds. It is advisable to measure the amount of urine secreted daily when sulphonamides are being administered. The reaction of urine and the presence of red blood cells should be regularly recorded. Sufficient quantities of fluids should be given to obtain a secretion of 1500 ccm (52 oz) of urine in adults; output below 1,000 ccm per day should be taken as a danger signal. Besides this, sufficiently large quantities of sodium bicarbonate, citrate or lactate, should be given by the mouth to keep the reaction of the urine alkaline. The reason for this is that the acetyl derivatives into which these drugs are partly converted in the blood are more soluble in alkaline than in acid urine. If hæmaturia is only microscopic it is not necessary to stop the drugs but calls for careful observation of urinary output thereafter. The findings of sulphonamide crystals in the urine of patients under treatment with these drugs suggest caution but the treatment need not be stopped if the urinary output is satisfactory and there are no signs of renal damage or obstruction. Differential leucocyte count should be made wherever

possible on the 3rd and 5th days after beginning of massive sulphonamide therapy; intensive treatment with these drugs as a rule should not be continued beyond the 7th day. If the severe infection from which the patient is suffering produces leucopoenia, sulphonamide treatment should not be withheld but a careful watch should be kept by doing frequent blood counts because of the risk of agranulocytosis which is one of the most dangerous sequels of sulphonamide therapy. This condition rarely makes appearance before the 14th day of the therapy. The total and differential white blood counts should be done at least twice a week after the 7th day. If the polymorphonuclear leukocytes fall below 50 per cent or if the total white cell counts rapidly falls below 4,000 per ccm, the treatment should be immediately stopped.

(2) Toxic reactions and side effects

Toxic reactions with this group of drugs whether mild or severe commonly occur and a close watch should be kept for them. The pathogenesis of these reactions is not clearly understood, some being due to the toxic action of the drugs while others are due to hyper-sensitivity. There may or may not be history of previous use of the drugs. Sensitiveness to these drugs occurs from previous use even many years previously. Sometime patients sensitive to one drug of sulphonamide group are not sensitive to another sulphonamide. As a rule toxic reactions appear after the drug has been used for several days but sometime a violent reaction may be produced immediately after the first dose.

Disastrous consequences from the use of these drugs if given judiciously are fortunately not common. There is no doubt, however, that increased number of people are acquiring hyper-sensitivity to sulphonamide drugs. Toxic reactions are more common with sulphanilamide, sulphapyridine and sulphathiazole and less with sulphadiazine. The pathological changes produced by sulphonamide drugs have been mostly worked out in animals. In a series of eight human fatal cases, besides changes in the kidneys, the chief lesions were multiple areas of local necrosis in the liver, lungs, bone marrow, spleen, adrenals, myocardium and lymphatic glands. Insterstitial myocarditis has been recorded from prolonged use.

Toxic effects, whether mild or severe, often occur with heavy doses and in some individuals even with small doses probably due to unsuspected idiosyncracy to the drugs. This is specially the case with disturbances of hæmopoietic system, *e.g.*, agranulocytosis whose onset may be very insidious and unless frequent blood examinations are made, the bone marrow may be seriously damaged before it is recognized. Such effects are, however, not common and often disappear when the drug is stopped. If, however, there is severe disturbance in the blood, blood transfusion may have to be immediately given.

The toxic effects produced by different sulphonamides are summarized in the following table modified from Medical Research Council Memorandum No. 10:—

(3) The more important toxic effects in detail

(a) *General.*—The patient may complain of malaise and discomfort or a feeling which he cannot exactly describe. There may be a combination of nausea, headache, tinnitus, giddiness, lassitude and intense mental depression. Such symptoms are more frequent with sulphanilamide and sulphapyridine and less with sulphadiazine derivatives. If the patient is kept in bed these complaints are not very troublesome but in case of ambulatory patients they become a difficult problem especially when patients are engaged in such trades as motor driving, etc., in which these are hazardous. Use of mild sedatives is sometimes helpful.

Reaction	Sulphanilamide	Sulphapyridine	Sulphathiazole	Sulphadiazine	Clinical significance
Nausea vomiting	Occasional	Common	Occasional	Uncommon	If severe discontinue drug
Dizziness and headache	Common	Common	Uncommon	Uncommon	Not serious
Cyanosis	Common early and late	Faint, common early and late	Uncommon	Rare	Not serious
Drug fever	Common (5th to 9th day)	Common (5th to 9th day)	Common (5th to 9th day)	Occasional	If serious discontinue drug
Rashes (dermatitis)	Occasional	Occasional	Occasional	—do—	—do—
Primary or acquired hypersensitivity, with fever, rashes, lymphadenopathy, etc.	Rare	—do—	—do—	Rare	—do—
Leucopoenia with granulocytopenia	Occasional (early or late)	Occasional (early or late)	Occasional (early or late)	Rare	—do—
Agranulocytosis	Occasional	Occasional	Rare	Very rare	—do—
Aplastic anæmia	Very rare	Very rare	Very rare	—do—	—do—
Purpurea with or without thrombo-cytopenia	—do—	—do—	—do—	—do—	—do—
Mild hæmolytic anæmia	Not infrequent (early or late)	Not frequent	Not frequent	Not frequent	Not serious
Acute hæmolytic anæmia (occasionally with hæmoglobinuria)	Rare	Very rare	Very rare	Very rare	If serious stop drug Give alkalis, transfusion of blood and plasma
Hæmaturia	Doubtful	Common generally early	Common generally early	Occasional	If microscopic, discontinue drug

Reaction	Sulphanilamide	Sulphapyridine	Sulphathiazole	Sulphadiazine	Clinical significance
Anuria with azotaemia	Not reported	Not uncommon (first 10 days)	Not reported (first 10 days)	Common	Stop drug
Acidosis	Occasional	Not reported	Not reported	Not reported	Give alkali
Marked leucocytosis	Rare; generally in presence of hæmolytic anæmia	Rare; generally in presence of hæmolytic anæmia	Not reported	Not reported	Not serious
Injection of sclerae and conjunctivae	Not reported	Reported	Not uncommon; may occur with rash and fever (5th to 9th day)	Rare	May be serious discontinue drug
Arthritis	Not reported	Not reported	Occasional	Not reported	
Ocular and or auditory disturbances	Rare	Occasional	Reported	Reported	May be serious discontinue drug
Mental disturbances	Occasional; occur early	Occasional; occur early	Rare	Rare	
Neuritis	Very rare	Very rare	Very rare	Very rare	May be serious discontinue drug
Hepatic damage	Occasional early or late	Occasional	Occasional	Not reported	—do—
Jaundice	Rare; with acute hæmolytic anæmia or hepatitis	Rare; with acute hæmolytic anæmia or hepatitis	Rare; with acute hæmolytic anæmia or hepatitis	Not reported	—do—
Splenomegaly	Occasional	Doubtful	Occasional	Rare	—do—
Psychosis and delerium	Uncommon	Uncommon	Very rare	Very rare	If severe discontinue drug

(b) *Nausea, vomiting and diarrhoea.*—Vomiting is the commonest and most troublesome symptom and frequently occurs with sulphapyridine. It is the result of irritant action by the drugs on the gastric mucous membrane to some extent, but not entirely. There is probably some central action also as vomiting occurs even when the drug is given by injection and may be very severe after comparatively small dosage. In most cases vomiting can be mitigated by omitting or reducing some doses, giving crushed tablets in milk or fruit juice or suspended in one dram of mucilage of tragacanth, and by keeping the patient on very light diet. Alkali or acid given at the same time as the drug may be helpful. Sometime five minims of tincture of opium given before each dose prevent it; 50 mgm of nicotinic acid by mouth with each dose may be effective. Glucose drinks are useful. Proper spacing of individual doses may also be helpful.

Vomiting is not an indication for stopping the treatment but if it becomes so severe that the drug cannot be absorbed, sulphonamide may be given parenterally. In severe infections, if vomiting occurs after sulphapyridine one of the other drugs may be given. Replacement of sulphapyridine by sulphanilamide or sulphadiazine may be effective.

Diarrhœa sometime occurs after administration of these drugs, but is not a serious problem. Appetite is generally lost when the drugs are being taken. Stomatitis rarely occurs after sulphathiazole.

(c) *Effects on the nervous system.*—Both the central and peripheral nervous systems may be affected but this is comparatively rare.

Central nervous symptoms are, psychosis with delerium, delusion, hallucinations, loss of memory and encephalomyelitis, the last named being a very serious complication due to inflammatory changes in the walls of cerebral vessels. It has occurred after small doses and probably is a rare idiosyncracy. Polyneuritis, optic neuritis, blindness, aphasia, stammering, meningeal signs and convulsions may occur. Mental aberrations, such as those simulating alcoholic intoxication, diminution of mental acuity, acute psychosis, wild delirium and maniacal states have been observed. Organic changes in the nervous tissues can only occur with very massive doses in experimental animals.

Peripheral neuritis is a rare manifestation and has been observed after use of sulphathiazole in large doses and for prolonged periods. The patients generally recover from nerve injury thus produced.

(d) *Cyanosis.*—It usually appears early in the course of treatment and is due to formation of methæmoglobin and rarely sulphæmoglobin, the latter a later stage of the former. The formation of methæmoglobin from hæmoglobin is a reversible process and methaemoglobin disappears soon after the drug is stopped. These pigments can be demonstrated in the blood and differentiated spectroscopically; as much as 30 to 35 per cent of hæmoglobin may be converted to methæmoglobin. Though alarming to the patients' relations, cyanosis is not of any great significance except in patients who are markedly anæmic and it is not an indication for stopping the drug. In severe anæmia and pneumonia, it may aggravate the disability by causing anoxæmia. The idea that sulphur containing fluids and drugs given concurrently predisposed to cyanosis has been shown to be incorrect.

When cyanosis is due to methaemoglobinaemia, it can be controlled by methylene blue, which accelerates the conversion of methæmoglobin into hæmoglobin. It may be given either by intravenous injections of 1-2 mgm per kilo body weight, as 1 per cent solution (10 to 20 ccm), or by the mouth in

doses of 1 to 2 grains (0.2 gm) when it acts in 3 to 4 hours. With this treatment cyanosis rapidly clears up but it may return after 24 hours. It can be kept down by giving methylene blue with sulphonamides from the very beginning. Methylene blue may, however, cause vomiting and diarrhœa, and when given by injection it is liable to cause tissue necrosis. When cyanosis is due to sulphaemoglobinæmia, methylene blue has no effect; unlike methæmoglobin, sulphæmoglobin is not readily convertible into hæmoglobin. Marked cyanosis is seen mostly after use of sulphapyridine and sulphanilamide, less after sulphathiazole and sulphadiazine derivatives and not at all after sulphaguanidine and succinylsulphathiazole.

(e) *Acidosis or ketosis.*—This is indicated by hyperpnœa with or without physical exertion and acid urine or in case of ketosis by smell of acetone in the breath, drowsiness, vomiting and acetone in urine. There is reduction of CO_2 combining power of the blood and increase in alveolar and blood CO_2. There is increased output of sodium in urine, increased alkalinity of urine and diminished excretion of ammonia. Hyperpnœa is due to excess of CO_2 in the blood due to inhibition of enzyme carbonic hydrase, which hastens dissociation of $H_2\ CO_3$ into CO_2 and H_2. For this inhibition $SO_2\ NH_2$ group occurring in sulphanilamide is essential and even with such concentrations as 3 to 4 mgm per cent, patients may experience difficulty when taking strenuous exercise.

In such cases large doses of sodium bicarbonate should be given and if ketosis is present glucose or sugar should be administered. Acidosis is minimised by giving bicarbonate of soda in doses equal to the drug with each dose, but such a practice is not desirable. Acidosis generally occurs after sulphanilamide, sulphapyridine, sulphadiazine, sulphathiazole and sulphaguanidine. Some degree of acidosis always occurs with these drugs though it may not be clinically evident. The loss of base during excretion may produce an alkaline urine and in some cases air hunger may actually be present.

(f) *Drug fever.*—This occurs during the administration of the drug or within 24 hours after the course has terminated. It occurs with all sulphonamides but more frequently when sulphanilamide or sulphathiazole is used than with others, but is frequent with sulphamezathine. If the drugs are given for the first time, it rarely appears before the 7th day of treatment but if previous courses have been given, it may appear much earlier even on the 1st day. Drug fever may be accompanied by chills, severe headache, vomiting, tachycardia, skin eruptions, cyanosis and collapse. Once an individual has had drug fever, it may recur months later following a single small dose of the compound and the succeeding reaction may be very violent. That is the reason why patients are to be questioned about previous doses. If fever is produced before six days after the administration of the drugs it is often an indication that other toxic symptoms including skin lesions, hæmolytic anæmia, etc., will occur later. If fever is definitely due to the drug it is a warning sign and the treatment should be temporarily stopped, Before restarting treatment, a small dose of the drug is given and if there is a sharp rise of temperature within 12 hours the case is unsuitable for sulphonamide therapy.

The incidence of drug fever increases during consecutive courses and is 36 per cent in a second course of sulphathiazole and 80 per cent in the third course. Temperature may go up to 107°F. It is easily distinguished from that occurring with the aggravation of the disease for it appears after the temperature has fallen and clinical condition is improving. Omission of drug brings down the temperature but if it does not, agranulocytosis should be suspected. Para-aminobenzoic acid is neither preventive nor curative of this condition.

(g) *Skin lesions.*—Different kinds of rashes, closely resembling exanthematous fevers occur fairly commonly generally associated with drug fever in the course of sulphonamide therapy between 8th and 12th days of treatment. The most common rash is a discrete maculo-papular measles-like eruption, distributed over face, neck, wrists and forearms. Urticarial and purpuric rashes have been reported.

The distribution of rash and sometimes a history of exposure to light suggest photosensitivity. The rash on fading may leave staining and a desquamation. Sometimes a finely macular general roseolar eruption like German measles or a scarlatiniform rash may be met with. The rashes appear late in patients who have received the drug for the first time but if the drugs have been given previously the rash may appear within four hours of a single dose. Sulphathiazole sometimes causes a condition very much like erythema nodosum. Even exfoliative dermatitis has been produced, resulting in death.

The treatment of these affections is to stop the drug and give large amounts of fluid; the rash often disappears in 24 hours. As the rash may be a prelude to serious complications, a leucocyte count should be immediately made. Patients under sulphonamide therapy should not be exposed to strong sunlight or to x-rays during the treatment as they may be photosensitive.

Eczematous eruption may rarely follow systemic use, but more often after prolonged local use. The eruption is at first local but later may spread to other parts especially those exposed to the sun. When local treatment is given, a careful watch should therefore be kept. Sensitivity to such eruptions, when developed, lasts for a long time extending to a year or more. Desensitisation is carried out by giving 0.125 gm of the drug twice daily. The eruption reaches its maximum on the 5th day and then fades; the dose is then doubled. If a further reaction occurs the dose is again decreased till eruption fades.

(h) *Effects on the blood.*—(i) *Leucopœnia.*—Both production of white and red blood cells may be profoundly affected by these drugs, the former being more common and ranging from slight depression to total absence of granulocytes. Mild degree of leucopoenia is not important unless it rapidly progresses to below 4,000 leucocytes per cmm. On the other hand, agranulocytosis is very important and it may be assumed to be present if the total leucocyte count is below 2,500 per cmm and total neutrophil below 1,000 per cmm.. The drugs should be immediately stopped and copius fluids given; in severe cases repeated transfusions of fresh blood are indicated. Agranulocytosis occurs both with sulphanilamide and sulphapyridine, more commonly with the latter, where larger quantities of the drug, usually 100 tablets, have been given. Rarely sulphathiazole, sulphadiazine and sulphaguanidine produce it, but it has not been reported with sulphamerazine or sulphamezathine. The condition starts usually after the tenth day of administration or after more than 30 gm of the drug have been taken. If administration of the drug has to be prolonged beyond seven days a leucocyte count should be done, or earlier if large doses have to be given. The drug should be stopped if granulocytes fall below 2,000 per cmm. If granulopoenia is not checked in the early stages, symptoms of agranulocytosis develop.

(ii) *Agranulocytosis.*—The clinical picture of agranulocytosis is often indefinite and may not be diagnosed without blood examination. The characteristic symptoms of agranulocytic angina do not often occur until shortly before death or may not occur at all. These are, rising fever, drowsiness, inability to sleep, sore throat, cough, vomiting and aching pains in the limbs. This may be followed by severe septicæmia, ulceration of the throat and sometime of the

mouth, rectum and vagina. This danger can be avoided by having frequent blood counts, and if this is not possible, by carefully watching the dosage. High total dosage is more likely to cause agranulocytosis than high concentrations in the blood at any time. For this reason, the drug should not be continued too long and should be stopped if no clinical improvement in the course of the infection is evident If the fever is subsiding and the general condition satisfactory after six days of therapy, sulphonamides may be continued for two or three days longer. If, however, there is no response in temperature and if other disturbing symptoms are present or in any case if more than fifty tablets have been taken or treatment extended over more than ten days, a blood count must be done at once. Even a transient leucopoenia may be a sign of a tendency towards depression of bone marrow.

Leucopœnia chiefly affecting polymorphonuclears is the essential feature. The lymphocytes are at first unaffected but later decrease and the monocytes follow the lymphocytes. The total leucocyte count may fall to 50 per cmm. Both red cells and hæmoglobin in later stages show a progressive decrease.

Treatment.—Such decrease of polymorphonuclears on which body immunity is dependent and whose complete absence for seven days is incompatible with life, necessitates swift and drastic action if life is to be saved. Blood transfusions should be given at once and injections of liver extract should be given. Small exposures to x-ray have been advocated. Para-aminobenzoic acid in doses of 0.3 gm every four hours along with pentanucleotide has been suggested and is said to give better leucocyte response in some cases. When severe neutropoenia has occurred once in a patient he should not be given sulphonamides again.

Treatment of agranulocytosis is directed to leucopoenia and secondary bacterial infection which is responsible for local pharyngeal lesions often fatal. Pentanucleotide is toxic and is not much used now. If it is to be used, the dose should not exceed 10-20 ccm intramuscularly at least four times a day for four or more days until the leuocytes increase and young forms appear in the blood. The best leucopœietic stimulant is pyridoxine. Vitamin B_6 which is issued in 20 mgm tablets for oral use and 50 mgm ampoules for intravenous use.As much as 125-200 mgm are given daily intravenously. The symptoms usually subside in 48 hours but the injections should be continued for 5 to 6 days. Recovery is heralded by a leukaemoid reaction. Folic acid has also been used but it is not so successful as in macrocytic anaemias. Glutathione, adenine sulphate, liver extract, yellow liver marrow extract have been tried. To deal with secondary bacterial infection penicillin is best; sulphonamides should not be used for this.

(iii) *Anaemia.*—(1) A slowly developing fall in red cells with a drop of 10 to 20 per cent in hæmoglobin is fairly common after sulphonamide drugs have been given for ten days. It disappears when these are stopped and is not necessarily a contra-indication to continuing treatment.

(2) Acute hæmolytic anæmia is a rare but dangerous complication and develops early (2nd to 6th day). It is common with sulphanilamide and occurs more frequently in children and negroes. This is due to idiosyncrasy rather than over-dosage and is usually preceded by nausea and headache for about 6 to 12 hours; then follows fever, jaundice, rapidly increasing pallor and shock. The liver and spleen are rapidly enlarged; plasma bilirubin is raised and plasma contains hæmoglobin, methaemoglobin and methæmalbumin; urobillinogen occurs in urine. In some patients the blood may be hæmolysed in one night; hæmoglobinuria may occur; the blood picture shows fall of red cells, leucocytosis

(100,000 per cmm) and reticulocytosis. When such conditions appear the drug is stopped and blood transfusion should be given immediately; alkalies and large quantities of fluids should be given by mouth or, if this is not possible intravenously, as in the treatment of black-water fever, to prevent blockage of renal tubules with blood pigments.

Mortality in this condition is very high despite active treatment; relapses occur when the patient appears to be recovering and sulphonamide is given again.

Minor degrees of anæmia not uncommonly occur after sulphonamide therapy and such cases should be treated with iron therapy.

(3) In view of the fact that blood transfusions have to be given it should be borne in mind that sulphonamides may produce alterations in blood grouping of an individual. This reaction is, however, rare.

(i) *Anuria, haematuria, etc.*—These are due to blockage of urinary passages by crystalline deposits of less soluble sulphonamides (sulphapyridine, sulphathiazole or sulphadiazine and to lesser extent with sulphanilamide). It is liable to occur when the secretion of urine is small owing to deficient intake of fluid or fluid is lost through vomiting or excessive sweating in hot climates. It also occurs if large doses of these drugs have been given by the mouth or intravenously and where there is partial obstruction in the urinary tract or toxæmia associated with acidosis is present. The site of blockage may be in the renal tubules or in the renal pelvis or ureters. This may occur within first 10 days and has been known to occur after 5 to 10 gm of the drug have been given. Hæmaturia does not seem to be related to dosage since it has occurred as early as 20 hours after the initial dose, or it may be delayed as long as six days. In severe cases it may be accompanied by oliguria and followed or preceded by anuria.

Briefly, renal failure may occur (1) in a acute hæmolytic anæmia; (2) by direct toxic effects of the drugs on the kidney; (3) formation of crystals in the kidney resulting in a mechanical interference to the flow of urine.

The signs present are pain in the region of the loins radiating to the groin or vague pain in the abdomen. There is also diminished output of urine proceeding to complete anuria. Hæmaturia, microscopic or macroscopic should be looked for if oliguria or anuria is present.

Prevention.—These conditions can be prevented if large quantities of fluids are given (4½ to 6 pints for an adult in 24 hours) to maintain a minimum urinary output of over 1,500 ccm or 52 fluid ounces in 24 hours, particularly when sulphapyridine, sulphathiazole and sulphadiazine are being given. The urine should also be kept alkaline to litmus by administration of alkalies. If renal pain or macroscopic hæmaturia or oliguria occur, the drug should be stopped immediately and copious fluids should be given unless there is complete obstruction. Heat should be applied to the loins by hot water bottles or short-wave therapy. Generally this gives relief but if anuria persists for 12 hours or if oliguria (less than 500 ccm or 17 ozs per day) persists for 23 hours, cystoscopy should be done and a ureteral catheter passed without delay. The catheter may encounter gritty obstruction in the ureters. The renal pelvis should be irrigated with warm 2.5 per cent sodium bicarbonate solution till the return of flow of urine is clear. The catheter should be left in position until urinary function is re-established.

In most cases hæmaturia is not a serious symptom and disappears 24 hours after the drug is stopped.. Sulphonamides should be given with caution in cases of renal impairment.

(j) *Effects on the liver*:—A few cases of acute yellow atrophy of liver have been recorded after heavy dosage for the treatment of gonorrhœa. It has also occurred after sulphanilamide has been placed in the peritoneum after operation. Sulphapyridine may remain in liver for 40 days after the drug has been stopped.

Hepatitis has occurred with sulphapyridine and sulphathiazole but not after sulphadiazine. the onset usually occurring after the third week of administration. The main symptoms are jaundice, enlargement of the liver and spleen and ascites. The condition may pass off or may be fatal. The prognosis is serious with previous cirrhosis and for this reason sulphonamides should not be prescribed in this condition. Hepatitis should be distinguished from jaundice and acute hæmolytic anæmia which occur in the first week.

Treatment consists in stopping the drug, giving glucose drinks and copious fluids. The diet should contain abundant carbohydrates and no fat; skimmed milk, boiled fish and eggs are given. Sulphanilamide has greater toxicity for the liver than sulphapyridine and this latter drug is more toxic than sulphathiazole. Sulphadiazine has the least toxicity and should be prescribed in all cases where there is impairment of liver function.

(k) *Arthritis.*—Sudden onset of pain may occur in the larger joints of the body especially after sulphathiazole and the skin over the joint may become red and swollen. One or more of the joints may become affected. Arthritis usually occurs in cases with fever and dermatitis and it has been mistaken for acute rheumatic fever. When the patient under treatment is suffering from gonorrhœal arthritis it may be confusing. It can, however, be distinguished by the fact that pain from the joint disappears when the drug is stopped.

(l) *Conjunctivitis and scleritis.*—Inflammatory reactions of the conjunctiva and sclera are sometimes seen after sulphathiazole therapy has been continued for several days, the symptoms resembling pink eye. There is sterile purulent discharge, burning and itching sensation and injection of blood vessels. The eye becomes normal after the drug is stopped. Administration of sulphathiazole on later occasions may or may not produce this reaction.

(m) *Thrombocytopoenic purpura* is a rare complication. Interference with blood grouping has not been definitely established though it is said to have occurred. There is no evidence either that administration of these compounds produces any changes in the motility or number of spermatozoa in the semen.

Summary of toxic and side effects:—

Nausea, vomiting, diarrhœa, stomatitis, black tongue, pyrexia, drug rashes, cyanosis, crystalluria, hæmaturia, oliguria, anuria with azotemia, acute nephrosis. Mild graunlocytopaenia, progressive granulo-cytopaenia, agranulocytosis; aplastic anæmia, mild hæmolytic anæmia, rarely with hæmoglobinuria, thrombo-cytopaenia with purpura; vitamin deficiencies. Encephalopathy and mental disturbances, toxic psychosis, Jaundice and hepatitis. Histologically necrotic lesions in liver and bone marrow, widespread eosinophilic infiltration, interstitial inflammatory lesions in heart, kidneys and liver; polyvasculitis in liver, kidneys, with necrosis of media of arterioles and small arteries.

10. Sulphonamides in Therapy

From point of view of the general practitioner, the difficulties of sulphonamide therapy, apart from its value in many conditions he has to treat, must be considered:—

(i) To be effective, massive doses have to be given from the beginning which are near to toxic doses and the medical practitioner who uses these drugs must be prepared to take the risk of discomfort and trouble to the patient accruing therefrom.

(ii) Doses have to be repeated every 4 to 6 hours day and night if adequate and effective concentrations are to be obtained and maintained in the blood. This necessitates a responsible person or a nurse to administer the correct dose regularly and at definite intervals. Untoward effects not infrequently arise which the physician should take note of and deal with without delay. The use of a well equipped laboratory and expert advice with regard to the condition of blood is required.

(iii) Apart from their serious toxic effects, the tendency of these drugs is to produce intractable nausea and often the anxious relatives want the treatment to be discontinued on this account, which increases the difficulties.

(iv) Cyanosis, although not a serious symptom and one which does not indicate discontinuance of treatment, is alarming to the patient and his relatives, especially in cases of pneumonia. The patient and his relatives wish to stop treatment with these drugs because they consider it serious.

(v) Haphazard treatment with inadequate doses and with interruptions, for such conditions as tonsillitis, common cold, mild cystitis, etc., is positively dangerous as there is danger of agranulocytosis and encephlomyelitis occurring leading to fatal results if a patient has idiosyncrasy to the drug.

(vi) Ambulatory patients should never be given tablets of these drugs without explicit instructions as to dosage and the length of time they are to be taken. Many deaths have occurred from overdosage in such cases as gonorrhoea and post-mortem examination has shown acute yellow atrophy of the liver, the symptoms preceeding death being intense vomiting, violence, irrationality and coma.

(vii) Sulphonamides are very potent drugs and their use should be reserved for patients in whom the effects can be carefully watched and controlled. Though hospitalization may not be absolutely necessary, the means to counteract the untoward effects should be at the disposal of the practitioner.

The practitioner should realise that there is always risk attached to adequate suiphonamide therapy. He should appreciate that formerly sulphonamide drugs were the only effective remedies available against bacterial infections. With the advent of the even more effective and practically non-toxic antibiotic penicillin and its availability in sufficient quantities, the indications for the use of sulphonamide drugs have been considerably narrowed down. The sulphonamides need only be used now in infections with organisms either nonsusceptible to penicillin or those only slightly sensitive to its action. In this connection the development of other antibiotics such as streptomycin and others, which may fill up the hiatus left by penicillin, is awaited with great and ever increasing interest. The practitioners should, therefore, make use of penicillin in all cases where it is possible to use it. The sulphonamides should only be used when these have to be used. (See section on antibiotic).

Complications after sulphonamides occur in 2.5 per cent and mortality rate is 0.2 per cent. Some degrees of visceral damage always occur; myocarditis has been found post-mortem. Unpleasant effects such as nausea. vomiting, headache and depression always occur. These drugs, therefore, should not be used unless there is definite indication. They should not be used in ordinary fever or conditions which react to other drugs. Sulphonamides should never be given in the treatment of mild complaints as these may produce drug sensitivity, which may prove fatal if the drug has to be used for serious disease later. Giant urticaria and fatal pulmonary œdema or aplastic anæmia may occur.

Overdosage is not common. High blood levels, such as 180 mgm per cent of sulphathiazole, 175 mgm of sulphanilamide, 170 mgm of sulphadiazine, and 65 mgm of sulphapyridine per cent are required to produce toxic effects in mice. Death in man usually results from urinary obstruction from deposition of crystals. Undue prolongation of treatment is harmful and gives rise to renal and hepatic damage. Seven days treatment is the maximum in a course. Toxic hepatitis occurs in the second week and agranulocytosis in the third week. If a more prolonged course is required on account of possibility of relapse, reduction of dosage for a few days usually prevents this.

Drug fever should be differentiated from exacerbation of infection which does not show such accompaniments as eruption, vomiting, etc. Use of sulphathiazole may produce temperature of 106-107°F. If sulphonamides have been previously given, drug fever may occur within a few hours after the first dose; this is likely to occur when the patient has fever and rash previously. Such reactions are common with sulphathiazole and sulphadiazine. Fever does not occur with sulphathiazole unless the drug has been given for more than seven days. When a second course is given 36 per cent get it, after a third course 80 per cent.

Under-dosage is likely to produce resistance of the organism. Adequate doses should always be given to produce quick response. It is best to follow a high loading dose by a short intensive course.

These drugs should not be prescribed to ambulant cases. They act better when the patient is resting in bed. Rarely acute psychoses develop quite suddenly. Dizziness of the head and sensitiveness of the skin to light is common.

Sensitisation dermatitis may occur from prolonged and local use. Appearance of eczema round the area of use is the first indication. It has very rarely occurred after systemic use.

The following drugs are likely to produce peripheral neuritis and should not be used:—

Sulphanilyl sulphanilamides, sulphanyl dimethyl sulphanilamide and methyl sulphathiazole.

A combination of sulphonamides with barbiturates before anaesthesia is contra-indicated.

11. Sulphonamides in Infectious Diseases

(1) Streptococcal infections

(a) *Haemolytic streptococcus.*—Streptococci have been divided into several groups. B-hæmolytic streptococcus (Group A, Lancefield) is responsible for most of the common infections in man. This group is fortunately very sensitive to the action of sulphonamides as also is group C. Groups B, F, G. H & K are unsusceptible but they are non-pathogenic.

The antibacterial action of sulphonamides was first demonstrated in infections with hæmolytic streptococci, and these organisms are amongst the most sensitive to the inhibitory action of the sulphonamide compounds. They are of the greatest value in rapidly spreading lesions where there is little necrosis, *e.g.*, cellulitis, lymphangitis, adenitis and erysipelas. In the two former infections, moderate dosage is generally sufficient, and may be gradually diminished after the temperature has been normal for 48 hours. Local collections of pus should be surgically drained and local application of sulphanilamide is helpful. In erysipelas, excellent results have been obtained with sulphanilamide, but it is preferable to employ sulphamezathine on account of its lower toxicity and higher potency. For complications, such as pyæmia, septicæmia or meningitis, sulphathiazole is the drug of choice. The treatment should be started with moderate doses, and these may be reduced to half after the temperature falls to normal. For streptococcal dermatitis of the face and ears, sulphathiazole wet packs (2 per cent aqueous suspension), or a 5 per cent sulphathiazole ointment, have given very good results.

Bacteraemia.—In streptococcal bacteraemia, the use of sulphanilamide has effected great reduction in the mortality and results are even better with the more potent sulphonamides. Sulphathiazole is perhaps the best drug to employ, and high blood levels should be maintained till blood cultures remain negative for at least a week. In addition any pus formations should be drained and blood transfusions given if there is any degree of anæmia.

Meningitis.—In meningitis caused by the hæmolytic streptococcus, sulphadiazine is the drug of choice, and intensive dosage should be continued till the temperature has been normal and the spinal fluid sterile for three days; thereafter the dosage may be reduced. The mastoid and cranial sinuses should be investigated for a primary infective focus, and sulphonamides should not be discontinued till such a focus has healed.

Erysipelas.—Sulphamezathine is preferable here on account of its low toxicity and sulphathiazole if such complications as septicaemia, pyaemia or meningitis occur. Dosage required is as for moderate infections and this is reduced to half when the temperature comes down and should be retained at this level for a week. Mortality has been reduced to about a quarter.

Tonsillitis and Rheumatic fever.—Usually tonsillitis is due to streptococcus Group A. Sulphanilamide in doses of 6.0 gm daily has given good results though this is doubted by many clinicians. Rheumatic fever is associated with streptococcal sore throat though its aetiology is unknown. Prophylactic doses of sulphanilamide reduce incidence of sore throats as well as of rheumatic relapses. The danger of agranulocytosis should however be borne in mind. Sulphadiazine is preferable in this condition because of its slow excretion.

Scarlet fever.—The value of sulphonamides is doubtful unless treatment is started at a very early stage.

Otitis media.—Otitis media should be treated from the beginning as a severe infection and sulphathiazole, sulphadiazine or sulphadimethyl-pyrimidine should be given in adults and children in dosage indicated in the table, though it is not necessary to give the first dose intravenously. It is rarely necessary to carry on the treatment beyond 7 to 10 days if rigorous treatment is started from the beginning. All the resistant cases should be treated with penicillin. Sulphonamide or penicillin therapy, however, does not abolish the need for surgical attention and careful local treatment is essential. Careful watch should be kept that drainage is maintained and that the mastoid is not involved.

Pneumonia.—In streptococcal pneumonia, the sulphonamides have not given such good results as in the case of pneumococcal infections, and the incidence of empyema has not been reduced although a reduction in mortality rates has been reported. In empyema, sulphonamides should be given in high doses and the effusion aspirated frequently. If, however, there is no improvement after a week's therapy, surgical treatment should be considered. Penicillin is preferable in such cases.

Osteomyelitis.—In both acute and chronic osteomyelitis of streptococcal origin, sulphonamides have proved very effective. Large doses of sulphathiazole should be given in acute cases, till the temperature has been normal for 2 or 3 days, after which the drug is continued at a reduced dosage till the sinuses have healed and the discharge is sterile; if the drug is stopped too early, infection may reappear. In chronic cases, moderate doses of sulphanilamide are often effective.

Puerperal fever.—In puerperal fever (infection generally A group, Lancefield), the best results are usually obtained with high blood concentrations of sulphanilamide, sulphadiazine or sulphathiazole. Sulphadiazine is considered by some to be the drug of choice as it is more pleasant to take, has a high potency, and adequate blood levels can be maintained with less frequent doses. The use of sulphonamides has reduced the general mortality from puerperal infections from over 25 per cent to less than 5 per cent.

Endocarditis, etc.—In infections, such as endocarditis, pericarditis, arthritis with pus, and brain abscess, the best results are obtained if the pus present is first aspirated. High blood levels should be maintained for 5 to 7 days and if the cultures are sterile the drug can then be continued at a reduced dosage till the patient has recovered.

Subacute bacterial endocarditis is often due to alpha haemolytic streptococcus which is insensitive to sulphonamides. Some cases are due to non-hæmolytic group some strains of which are sensitive. It may rarely be produced by *H. influenzae* or staphylococcus.

The sulphonamide drugs have been extensively tried in subacute bacterial endocarditis, and on the whole, results have not been encouraging. It would appear that the low penetration of the sulphonamides through the valvular vegetations is the major difficulty. The drug reaches the organisms embedded inside the vegetations in only very low concentrations, and the bacteria are thereby enabled to develop a certain degree of resistance to these drugs. There is thus generally an initial response to these drugs, the fever subsides and the blood culture becomes negative; the organisms, however, reappear in the blood stream, symptoms return, and the outcome is generally fatal. Recently results with high dosages of penicillin have given promising results (see sections on antibiotics) and it is likely that the use of sulphonamides in this disease will be given up in favour of the more active and less toxic antibiotic agents. Should sulphonamides be used, very high blood levels should be rapidly attained, and maintained over prolonged periods. Sulphamezathine can be given in high doses without the fear of renal complications, and is generally well tolerated over prolonged periods. An initial dose of 8 gm intravenously followed by 2 gm 4-hourly by mouth is the dosage generally advised. Sulphonamide therapy should also be supplemented by pyrexial therapy and for this purpose.

(b) *Non-haemolytic and anaerobic streptococci.*—These include the majority of the viridans group and "Indifferent" types of streptococci, as well as those having the characters of enterococci. All these are entirely insusceptible to sulphonamides as shown by in vitro tests and by clinical trials. Sulphonamides should not be used in these infections and if they are, they should be discontinued after three days if there is no response and treatment should be carried out with penicillin.

Peritonitis.—Primary peritonitis of streptococcal origin occurs in children and secondary peritonitis of metastatic origin in streptococcal diseases. Sulphonamides may be used both as a curative measure and prophylactically in deep penetrating wounds of the abdomen or after operation on a hollow viscus (anastomosis, etc.), as a supplement to operative measures. For collections of purulent exudates which may occur, systemic therapy does not give sufficient concentration locally and local application is necessary; sulphonamides in adequate quantities are introduced at or after the surgical operation. Sulphadiazine is the compound usually preferred on account of its slow absorption and low toxicity if penicillin is not available.

(2) Staphylococcal infections

Staphylococci are not very sensitive to the sulphonamide compounds and in general sulphonamides should not be relied upon in severe staphylococcal infections if penicillin is available. The consensus of opinion is that, especially in acute osteomyelitis, cavernous sinus thrombosis, severe carbuncle, meningitis, septicæmia, endocarditis and pneumonia due to staphylococci from whatever focus, penicillin treatment should be started immediately. Sulphonamides should only be used in these conditions if penicillin is not available. The drug of choice for use in staphylococcal infections is sulphathiazole, which is the most potent of these compounds.

Osteomyelitis.—Good results have been obtained in the treatment of acute staphylococcal osteomyelitis with the use of sulphathiazole. The necessity for operative intervention is often avoided, and the duration of the disease is considerably reduced if the operation of drilling has to be done. Blood levels of 5 to 7 mgm per cent should be maintained for 2 to 5 weeks and if surgical drainage is performed, sulphathiazole should be packed into the cavity. In chronic osteomyelitis, the use of sulphathiazole for 2 days before surgical operation is advisable as a prophylactic measure against bacteraemia. The cavity should be packed with sulphathiazole powder at the time of operation and sulphathiazole should also be given by mouth for several days after.

Boils and Carbuncles.—For the treatment of boils and carbuncles, sulphathiazole should be given by mouth in moderate doses, and also applied locally to the wound when an incision is made. This reduces the tendency to bacteraemia which is not an infrequent complication in such cases.

Pneumonia.—The value of sulphathiazole or sulphadiazine has been demonstrated in staphylococcal pneumonia. Sulphathiazole may be given in large enough doses to maintain blood concentrations of 5 to 10 mgm per cent; if empyema or lung abscess develops, these should be drained, and a 5 per cent suspension of sulphathiazole instilled locally into the cavity. Penicillin is, however, far more effective in the treatment of staphylococcal pneumonia, and if available should be employed in preference. The incidence of empyema is also considerably reduced.

Meningitis.—In staphylococcal meningitis, sulphonamides should be used only if penicillin is not available; a number of cures have been reported following their use. Sulphathiazole is the drug generally employed, and blood level of above 5 mgm per cent should be maintained for four days; afterwards this may be slowly reduced. Prolonged administration of these drugs is required because of the chronic nature of the disease, and frequent lumbar punctures are usually necessary. Sulphadiazine is reported to be more effective for experimental staphylococcal meningitis in animals, on account of its ready diffusibility into the spinal fluid.

In staphylococcal septicaemia sulphathiazole is said to have given beneficial results, but this is doubtful; penicillin is preferable in this condition. If sulphonamides have to be used, an initial intravenous dose should be given followed by intensive oral therapy to prevent resistance developing in the organism.

Tropical pyomyositis.—This is said to be a staphylococcal infection and sulphapyridine is reported to shorten the course of this disease.

(3) Pneumococcal infections

In their sensitivity to the sulphonamides, pneumococci stand mid-way between the highly susceptible organisms such as the haemolytic streptococcus and the gram-negative Nisseriae and the comparatively less sensitive staphylococci. Sulphanilamide is useless in pneumococcal infections. Sulphadiazine and its homologues, and sulphathiazole are the most effective, and sulphapyridine is also effective but should be used only when the others are not available.

Lobar pneumonia.—In lobar pneumonia, sulphapyridine was the first of the sulphonamide drugs which was found to be effective. It was extensively used with great benefit and a striking reduction in the mortality rate. This drug, however, produces frequent undesirable effects (nausea, vomiting, cyanosis and renal symptoms) and has now been superseded by sulphathiazole, sulphadiazine and sulphamezathine, which are not only more effective but do not produce untoward toxic effects to the same extent. Moderate blood concentrations usually yield satisfactory results, and are maintained till the temperature has remained normal for 24 hours. Thereafter the dose may be reduced by half, and continued for at least three days more. If the drug is stopped too early, the patient may get a relapse. The incidence of pneumococcal empyema has considerably decreased with the advent of sulphonamide therapy, but sterile pleural effusions often occur.

B. Friedlander pneumonia.—In *in vitro* experiments, *B. friedlander* has been shown to be susceptible to the more powerful sulphonamides, sulphathiazole being the drug of choice in this respect. In clinical practice, however, sulphapyridine and sulphadiazine are found to be more effective, and the chronic Friedlander pneumonia which normally takes about 6 weeks to cure, has been controlled in from 1 to 3 days with the use of these drugs. In virus pneumonia, sulphonamides are without effect.

Meningitis.—Before the advent of the sulphonamides, pneumococcal meningitis was almost invariably fatal. The use of sulphonamides effected considerable reduction in mortality, and penicillin has given even better results and should be administered by the intrathecal route if possible. Of the sulphonamide compounds, sulphadiazine is the one to be preferred on account of its ready diffusibility into the spinal fluid. Sufficiently high doses should be given to maintain blood levels at about 20 mgm per cent and for this 5 gm of the sodium

salt should be injected intravenously as the initial dose, followed by 1 gm every 4 hours. This dosage should be continued for at least 7 days after the spinal fluid has become sterile, and then the administration should continue with a reduced dosage for another week.

(4) Meningococcal infections

Meningococcal meningitis.—The meningococcus is even more susceptible to the action of sulphonamides than the hæmolytic streptococcus, and sulphonamide therapy should be started as soon as the clinical diagnosis is made. Sulphathiazole, sulphadiazine or sulphamerazine, sulphapyridine and sulphanilamide are all effective. Sulphamethazine has low penetrability into the cerebrospinal fluid and is not recommended. Sulphadiazine is the drug of choice in severe cases and the initial dose of 5 gm should be given by the intravenous route. Intrathecal injections of the sodium salts of sulphonamide compounds should never be given as they set up irritation. In patients who are unconscious or are unable to swallow, the drug may be given by the intramuscular route or by continuous subcutaneous drip. In such cases liberal amounts of fluids should also be given by the parenteral route to ensure an adequate urinary output.

There appears to be no advantage in using serum together with sulphonamides. In fact in a large series of cases, sulphonamide therapy alone gave better results than did the cases with combined sulphonamides and serum therapy. After the first diagnostic lumbar puncture, it is unnecessary to perform further punctures unless there are signs of increased intracranial pressure, or the patient does not respond to treatment. Penicillin is very effective in the treatment of this disease, but as response to sulphonamides is so good, penicillin is not generally indicated unless the patient shows signs of intolerance to sulphonamides. Penicillin wherever possible, should always be given by the intrathecal route.

The prophylactic use of sulphonamides in doses of 2 to 3 gm daily for 2 to 3 days has been found to reduce both the incidence of cerebrospinal fever and the carrier rate in epidemics of meningococcal meningitis. The mortality rate in this condition has been reduced from 50 to 70 per cent to 1 to 9 per cent.

Chronic meningococcal septicaemia responds rapidly to sulphonamide therapy and the temperature comes down on the second day of treatment.

Purulent meningitis due to pneumococcus, staphylococcus, H. influenzae, etc., is generally resistant to sulphonamide treatment. Sulphathiazole, sulphadiazine or sulphadimethylpyrimidine may, however, be given in dosage as required for servere infections; the course should be extended and the initial dose should be given by the intravenous route. Frequent examinations of cerebro-spinal fluid should be made so that relapses particularly common with *H. influenzae* may be detected and treated. If penicillin is available it should be given intrathecally as even *H. influenzae* is only relatively more resistant to penicillin.

(5) Gonococcal infections

Gonorrhoa.—The gonococcus is very susceptible to the action of sulphonamides, and by using the bone marrow culture technique it has been established that the susceptibility of the gonococcus to sulphonamide compounds was in the following ascending order: Sulphanilamide, sulphapyridine, sulphadiazine and sulphathiazole. Sulphathiazole was found to be effective in concentrations of 2 mgm per cent. During the last few years, however, the use of penicillin has so revolutionised the treatment of gonorrhœa, that it is now generally agreed

that penicillin should be employed in preference to the sulphonamides not only for the treatment of sulphonamide resistant cases, but for all cases of gonorrhœa. A comparatively small amount of penicillin, given by a few intramuscular injections in a single day, will cure over 90 per cent of all cases of uncomplicated gonorrhœa, and the remainder are curable by further penicillin treatment.

For the sulphonamide treatment of gonorrhœa, sulphathiazole and sulphadiazine are the drugs of choice, and suggested dosages for adults are 5 or 6 gm a day given for 5 days. Large quantities of bland liquids should be given during this period. With such a regime the great majority of cases are rendered non-infectious as quickly as possible, and there is a minimum amount of subjective discomfort. No form of local treatment is necessary in males, but in women, simple vaginal douching with a warm potassium permanganate solution, 1 in 8,000 to 1 in 16,000, is generally advised. About ten per cent of cases are usually found to be resistant to sulphonamide therapy inspite of maintainance of adequate blood and urine concentrations. Such cases are believed to be due to the presence of a sulphonamide inhibitor in the patient's serum; and they respond rapidly and effectively to penicillin therapy.

Gonococcal arthritis and gonorrhoeal ophthalmia neonatorum respond well to the sulphonamides. For gonococcal arthritis, adequate blood levels should be maintained for 1 to 2 weeks and if no response is obtained, artificial pyrexial therapy should be combined with the treatment. In ophthalmia neonatorum sulphathiazole in doses of 0.25 gm, or sulphamezathine 0.15 gm for every 7 pounds body weight should be given 4-hourly for 5 days. Local application of sulphonamides does not improve the results and is unnecessary. Penicillin applied locally is more effective in these conditions.

Vulvo-vaginitis of gonococcal orgin in children reacts well to sulphathiazole.

Excellent results have been obtained from the use of sulphonamides in the prophylaxis of gonorrhea. In a series of 1,000 cases, where 4 gm of sulphathiazole were given on the day following exposure, only one case developed the disease as against 79 persons in a control group.

Non-specific urethritis due to hæmolytic streptococci and Bact. coli which is communicable, may occur; it reacts to sulphonamides. It may be borne in mind that it may only be a symptom of a serious lesion in the upper urinary tract.

(6) Bact. Coli infections

In the treatment of Bact. coli infections of the urinary tract, both methenamine and mandelic acid are useful provided the urine is sufficiently acid, but the sulphonamides are effective either in acid or alkaline urine. In addition to Bact. coli infections, the majority of the other organisms responsible for urinary tract infections, such as hæmolytic streptococci, *Staph, aureus, Proteus vulgaris, Aerobacter aerogenes, Ps. pyocyanea,* etc., are also sensitive to the sulphonamides. *Str. faecalis* infection does not respond, but this can be eradicated by the use of mandelic acid. In clinical practice, excellent results have been obtained following the use of sulphonamides, and most of these compounds possess adequate or therapeutic potency against Bact. coli infections. Sulphathiazole, sulphadiazine or sulphamezathine should be given in moderate doses till the urine has become free from pus, after which the dose may be reduced and continued till the urine has remained sterile for three days. No undue restriction of the fluid intake should be practiced, as besides being dangerous, this has no particular advantage.

Alkalis are generally given during the course of treatment to minimize the chances of precipitation of the drug in the renal tubules. There is danger of organism becoming drug resistant if inadequate doses are given.

(7) Typhoid, paratyphoid and salmonella infections

The sulphonamide compounds, while bacteriostatic to these organisms in vitro, have not proved of any value in extensive clinical trials. In typhoid fever, the bacteraemia remains unaffected, the bacilli continue to be excreted in the urine, and the incidence of complications is not diminished, nor are typhoid carriers affected by any of the sulphonamide compounds yet available. The position with regard to paratyphoid and salmonella infections appears to be at present much the same.

(8) The Dysenteric Infections and Cholera

Bacillary Dysentery.—The bacillary dysentery infections are mostly localised intestinal infections, and it would appear logical to employ drugs, which while exerting powerful local effects against the infecting organisms in the intestine, are not readily absorbed into the system and so produce toxic effects. Sulphaguanidine is fairly rapidly absorbed, but the absorption is very incomplete and as a result, high concentrations such as 400 to 4,000 mgms per cent, are found in the fæces after an oral dose of 6 gm. This drug is therefore especially suited for use in such infections.

In the treatment of acute bacillary dysentery in adults, sulphaguanidine in doses of 3 gm three or four times a day for three days, followed by a gm twice daily for four days, has given very satisfactory results. For severe infections, the above unit of dosage may be increased to 5 gm. For children the dosage may be calculated on the basis of 0.05 gm per kilo body weight 4 hourly during diarrhoea, and then 0.01 gm per kilo 8 hourly for one to three days more. The duration of a course of treatment should not exceed 14 days, though the course may be repeated after a suitable interval if necessary. For chronic cases, a course of 5 gm 8 hourly for five days, followed by 2½ gm 8 hourly for another five days is recommended. Sufficient fluids should be given to ensure a minimum urinary output of 1500 ccm in 24 hours, to combat dehydration and to minimise the risk of blockage of the renal tubules.

A survey of recent literature on the subject shows that in the treatment of the acute stages of bacillary dysentery, present opinion favours the use in moderate dosage of one of the more easily absorbed preparations, such as sulphadiazine, sulphamezathine or sulphapyridine till the fever and diarrhoea have subsided. The dosage is then reduced to half, and continued at this level till rectal swab cultures become negative, but not for more than 7 days.

Succinyl sulphathiazole and phthalyl sulphathiazole are both slightly absorbed from the gut and are used in average doses of 20 gm and 12 gm respectively daily in divided doses at 3 hour intervals. Although sulphaguanidine is absorbed in higher proportions than these drugs, no cases of anuria have occured even in dehydrated patients in doses of 6 to 8 gm initially followed by 3 to 4 gm four hourly until the stools become normal. Sulphadiazine is largely used in America in doses of 2 gm initially followed by 1.0 gm four times daily.

Phthalysulphathiazole was inferior to Sulphanilylbenzamide but both these drugs were superior to Sulphaguanidine in all respects, particularly against Sonne, Schmitz, Boyd and Newcastle organisms. Bose and Ghosh (1945) treated 21 cases of bacillary dysentery with successful results. Treatment was

started with Sulphanilylbenzamide tablets (0.5 gm. each). For the first two days 2 tablets every 4 hours were given. For the next 4 days 4 tablets daily given. Usually 6 days treatment was quite sufficient, excepting Shiga cases, which required the drug a little longer. No accessory treatment was given except glucose and sodi bicarb drinks. On an average, 28 tablets were necessary for one adult patient. Thus the result of therapy with sulphanilylbenzamide was found satisfactory. Improvement started within 24 hours of treatment and the patients were fit to resume their work after the 4th day. Subsequent stool cultures of all the cases once a week for 3 months showed negative results. No relapse occurred in any of the case within 6 months of observation period.

Shiga dysentery infections are generally severe and it is then advisable to give the drugs in the maximum dosage (5 gm 3 or 4 times daily). These cases usually respond very well. Tenesmus and abdominal pain is relieved in a day or two, the temperature falls to normal in 1 to 3 days, and the stools return to normal in 5 to 6 days. Response is more rapid the earlier the treatment is begun. In very severe and fulminating cases it is advisable to supplement sulphonamide therapy with 50,000 to 100,000 units of anti-dysenteric serum.

Flexner dysentery infections respond rapidly and effectively to the moderate doses of sulphonamides suggested above and the pathogenic organisms are eradicated from the fæces after a few days of treatment. Convalescent carriers and symptomless contact carriers may generally be cured of their infections with a short course of sulphaguanidine.

Bact. sonne infections are clinically generally very mild and the aim of therapy is directed towards curing the convalescent carriers, and thus reducing the danger of spread of the infection among the community. Such carriers do not respond well to sulphaguanidine. Sulphasuccidine and pthalylsualphathiazole have been reported to be more effective, though not entirely satisfactory. Recently initial results with the new compounds, sulphanilylbenzamide and sulphanilamidobenzamide have been more promising. (see also section on bacillary dysentery).

Cholera.—Chopra and co-workers first used sulphaguanidine in the treatment of cholera in 1941 and obtained favourable results. The doses they used were, however, small (1 gm initially, and 0.5 gm 6 hourly for 72 hours). Huang in 1944 obtained very much better results with larger dosages (3 gm of sulphaguanidine initially followed by 1 gm every two hours for 6 hours, and then 1 gm 4 hourly for 1 to 2 days). It is advisable to use sulphaguanidine in the scale of dosage recommended for bacillary dysentery. In a recent series of cases (1945), using sulphadiazine (1 gm 4 hourly for the first day, and 2 gm 4 hourly for the next 2 days) a mortality of only 1 per cent was achieved as against a mortality rate of 38 per cent in a control group. Equally good results have been reported using sulphaguanidine in a number of series of carefully controlled cases at the School of Tropical Medicine, Calcutta, and elsewhere in India. Reports on the use of succinylsulphathiazole and pthalylsulphathiazole are not yet available and should be of great interest in view of the successful use of these drugs in other intestinal infections. It is of the utmost importance to bear in mind that the use of the sulphonamide drugs in cholera is likely to involve serious risk of urinary complications unless an adequate urinary output has been established before commencing chemotherapy. The use of the usual supportive therapy consisting of intravenous hypertonic saline and plasma solutions together with oral stimulants, to offset dehydration, emaciation and circulatory collapse, are absolutely necessary adjuncts to any chemotherapeutic measures employed. The value of supportive blood plasma treatment must also be stressed.

It should be remembered that coliform organisms are normal intestine symbionts and are concerned with Vitamin synthesis: The intestinal sulphonamides have remarkable effect on these and after their administration for a week or so, deficiencies of riboflavin (B^2) nicotinic acid, thiamine and vitamin K have been observed and a sprue-like syndromc may be produced. Black tongue occuring in nicotinamide deficiency has been observed after the use of sulphonamides as well as after oral use of penicillin. The purpuric rash which sometime occurs improves with intramuscular injections of Vitamin K.

(9) Other Infections

Plague.—The protective effect of the sulphonamides against plague were first demonstrated when Durand (1939) found that sulphapyridine could protect mice inoculated with 10,000 times the fatal dose of *pasteurella pestis.* Sokhey and Dikshit (1940) using sulphathiazole cured 85 per cent of mice infected with a fatal inoculum, even when treatment was started 48 hours later. They found that this drug was more potent than sulphapyridine, and gave as good results as the most potent serum. Wagle, Sokhey, Dikshit and Ganapathy (1941) in a large series of cases of bubonic plague, obtained mortality rates of 28 per cent using serum alone, 24 per cent with sulphapyridine, and only 15 per cent using sulphathiazole. Even better results have been obtained with a combination of serum and sulphonamide therapy. Sulphathiazole or sulphadiazine should be employed in the treatment of plague, in the same dosages employed as for other severe infections. This is combined with streptomycin on the basis of fatal daily dosage of 0.04 gm per kilo divided into 8 parts given intramuscularly day and night.

Haemophilus influenzae infections.—In pneumonia due to *H. influenzae,* the sulphonamides are not of much value, but in meningitis due to this organism, whereas previously the mortality rates were about 90 per cent in the ordinary age groups, and 100 per cent in infants under two years of age, treatment with sulphonamides combined with immune serum, has effected remarkable reductions in the mortality from this disease. In a large number of cases so treated, the mortality in the ordinary age groups was practically abolished, and deaths were almost confined to infants under 8 months of age. Sulphadiazine is recommended to be given in doses of 0.1 gm per kilo initially, a similar dose after 4 hours, and then a reduced dosage of 0.1 gm per kilo per day is continued till the spinal fluid has remained sterile for one week. 25 ccm of serum is given intrathecally and 15 to 75 ccm intravenously. Lumbar puncture is required to be performed daily.

Chancroid (Soft sore).—The prophylactic value of the sulphonamides in preventing chancroid in persons experimentally inoculated with virulent cultures of *H. ducreyi* has been demonstrated. Administration of 2.5 to 5 gm of sulphathiazole a day for two days, starting an hour after the inoculation, prevented the development of a chancroid in practically all the cases. Good results were also obtained by powdering the area with a sulphonamide powder. Sulphonamide or sulphathiazole ointment was less successful. The administration of 2 gm of sulphathiazole before exposure to risk and twice afterwards, was found to reduce the incidence of chancroid from 5.2 to 0.6 per cent

For the treatment of chancroid, sulphathiazole, sulphadiazine, sulphapyridine or sulphanilamide, may be given in doses of 4 gm a day, up to a total of 30 gm or till healing occurs. Buboes often subside without surgical treatment, and resolution may be aided by bathing the lesions in warm saline, and applying iodoform powder.

Tuberculosis.—Experimental work with the sulphonamides in tuberculosis has given conflicting results. Animal experiments, largely with guinea pigs inoculated with tubercle bacilli, have given negative results in the hands of some investigators, while others have reported retardation in the progress of the lesions.

Actinomycosis.—Rapid response of cases of actinomycosis to therapy with the sulphonamides have been reported by some observers while others have failed to obtain such success. Actinomycosis often responds in a striking fashion to penicillin treatment, and this drug may well prove to be the drug of choice. If it is decided to use sulphonamides, one of the more active preparations, such as sulphathiazole or sulphadiazine should be employed in high dosages as for severe infections.

Diphtheria.—Diphtheria does not respond well to sulphonamides and anti-toxin should be relied upon for treatment. Thomas (1941), however, found sulphathiazole snuff to be of value in diphtheria carriers, the organisms being eradicated in about 60 per cent of cases.

Anthrax.—The response of anthrax to sulphonamides is uncertain. Gold (1942) in a comparative series of 63 cases, treated 21 of these with immune serum alone, with a mortality of one, and 39 of the cases with sulphonamides alone, without a single death. He concluded that sulphonamide therapy was an adequate substitute for immune serum, and was more effective than neoarsphenamine. A combination of penicillin with one of the diazines is more effective.

Cutaneous anthrax appears to respond well to the sulphonamides.

Tetanus.—Sulphonamides appear to have no prophylactic value against tetanus, and tetanus anti-toxin should always be used where necessary. Bills, Hond and Smith (1941) succeeded in controlling experimental tetanus in mice with sulphathiazole and some measure of success has also been obtained in developed human cases of tetanus.

Gas gangrene.—The most effective prophylactic measure against gas gangrene is early and efficient surgical treatment. In wounds which are liable to develop gas gangrene such as extensive lacerations, deep penetrating wounds containing contaminated foreign bodies, and wounds where primary operation has been delayed, sulphathiazole should be applied locally in addition to the addministration of serum. Small doses of sulphathiazole should also be given internally, when gas gangrene has developed. The drug should at first be given intravenously, and the maximal blood levels should be maintained before surgical interference is attempted. Marfanil (amino-methyl benzene sulphonamide) is more active than sulphathiazole against *Cl. welchii*, and is successfully employed for local application to wounds.

Brucella infections.—While the brucella organisms are sensitive to the action of sulphonamides in vitro, results in cases of human brucellosis have been uncertain and conflicting. Initial results showed promise, but then relapses were found to occur in most cases. Treatment is most effective in acute cases of only a few weeks duration, as also in cases with bacteræmia or a high blood agglutination titre. Sulphadiazine is the drug of choice, as prolonged dosage is usually necessary. An initial dose of 4 gm is followed by 1 gm 4 hourly for 10 to 12 days. A second course may be given if necessary after an interval of several weeks.

(10) Rickettsial and Virus Infections

Virus infections.

Lymphogranuloma inguinale.—Virus infections are not as a rule benefitted by sulphonamides, but the virus of lymphogranuloma is susceptible. The drug, however, appears to act in a different way to bacterial infections, and the virus appears to persist in the body in an attenuated form. As against the normal duration of 6 to 8 weeks in untreated cases, in those treated with sulphonamides, the adenitis and proctitis clear up in 4 to 5 weeks. The Frei test, however, continues to be positive after cure, indicating that the virus still persists in the tissues. Sulphadiazine or sulphapyridine are the drugs generally employed in dosages of 5 gm daily for 5 days, followed by 3 gm daily for 6 to 12 weeks. Proctitis responds well to treatment even when stricture has developed.

Granuloma venerium.—In this condition antimony treatment combined with sulphonamides gives beneficial results if the disease is not very chronic.

Other virus infections.—Sulphonamides have no effect on the virus of influenza, but as it is often associated with bacterial infections, such as streptococci, staphylococci, etc., which are susceptible to sulphonamides; it may have some beneficial effect in preventing complications. Herpes zoster is not affected by sulphonamides.

(11) Affections of the Eye

Conjunctivitis.

Conjunctivitis responds well to 2.5 to 10 per cent solution of sodium sulphacetamide instilled at intervals varying from 2 to 8 hours according to the severity of the infection. Inclusion conjunctivitis, a virus infection which ordinarily runs its course for 4 to 6 months, usually heals in 2 to 3 weeks following the use of a 5 per cent sulphathiazole ointment.

Corneal ulcers.

Corneal ulcers generally respond well, and a 20 to 30 per cent solution of sodium sulphacetamide should be instilled into the eye every 2 hours in severe cases, and at correspondingly less frequent intervals in the less severe infections. In infected corneal abrasions such as are commonly seen in miners and industrial workers, good results are obtained with the application of sodium sulphacetamide powder after cocainisation of the eye.

Blepharitis.

For blepharitis, the application of a 2.5 to 10 per cent sodium sulphacetamide ointment, one to four times daily, is recommended. Sulphathiazole ointment also gives good results. Where the use of an ointment is not suitable a 10 to 30 per cent solution of sodium sulphacetamide may be applied as a paint to the eyelash roots after removal of the crusts.

Trachoma.

In trachoma, the sulphonamides appear to exert a powerful beneficial action in the clinical disease, the progress of the disease being arrested in about 90 per cent of the cases. Complete recovery, however, is not attained and it appears that the drug acts on the secondary invading organisms, rather than on the trachoma virus itself. Trachomatous tissues retain their infectivity to monkeys even after several hours soaking in sulphanilamide solutions. Sulphanilamide is recommended in doses of 3 gm daily for 10 days, and then a reduced dosage for a further two weeks. A saturated solution of sulphanilamide (0.8 per cent) may also be applied locally several times a day.

(12) Otorhinology

The course of acute *otitis media* is generally greatly cut short, and the discharge clears up rapidly. The disease should be treated from the start as a severe infection, and sulphathiazole, sulphadiazine or sulphadimethylpyrimidin

should be administered in dosages suitable for severe infections. Sulphonamide treatment is rarely required to be continued beyond 7 to 10 days. All resistant cases should be given a course of penicillin, and indeed many consider it preferable to use penicillin from the start. It must, however, be cautioned that there is a real danger in using sulphonamides indiscriminately in this condition, as sometimes progressive lesions in small undrained foci may be obscured. and a false sense of security may delay urgent surgical intervention. A number of cases have been reported, where the patients were rapidly relieved of all symptoms after sulphonamide therapy, only to develop brain abscess after a varying latent period.

In acute mastoiditis, treatment is on the same general lines as above, and surgical interference is frequently rendered unnecessary by the use of sulphonamides in high dosage continued till the post aural swelling and tenderness have disappeared, and the discharge markedly diminished. Dosage is then continued at a reduced level for a week after all signs of the disease have disappeared.

In *chronic mastoiditis,* encouraging results have been obtained, and after mastoidectomy, sulphanilamide or sulphathiazole powder or paste applied locally, hastens the healing process.

In acute sinusitis, sulphonamides have given exceptionally satisfactory results. Sulphamezathine or one of the other less toxic compounds should be employed with an initial dose of 4 gm followed by 1 gm every 4 hours till the temperature is normal. Thereafter a reduced dosage is continued for another 2 to 3 days. If suppuration occurs, surgical drainage must be performed.

(13) Diseases of the Skin

For a detailed discussion of the value of the sulphonamide compounds, in skin diseases reference should be made to the section on diseases of the skin. Only a brief summary of the general value of sulphonamides in diseases of the skin need be given here. Sulphonamides may be given by mouth or applied locally in the form of powders, ointments, creams and lotions. In lesions with much exudate, sulphonamides should be mixed with an oil in water emulsion as this readily mixes with the exudate. In dry lesions, water in oil emulsions are to be preferred. Simple mixtures of the drugs with soft paraffin are not very effective, as the drug is not readily liberated. High concentrations possess no advantage over low concentrations, and are more likely to cause skin sensitivity. Sulphanilamide, sulphathiazole and sulphadiazine penetrate the skin to about the same extent but sodium sulphacetamide has a much greater penetrating power.

Impetigo responds very well to a 5 per cent suspension of sulphathiazole in an oil and water emulsion, the lesions resolving in half the time necessary with ammoniated mercury preparations. In very severe cases sulphathiazole may be given by mouth as well. Drug therapy should not be continued beyond seven days to minimise the liability to drug eruptions. Favourable results have also been reported in erysipeloid.

The response is also favourable in sycosis barbae, infantile eczema, eczemoid dermatitis, pyoderma gangrenosum, pyogenic granulomata, and molluscum contagiosum.

Dermatitis herpetiformis responds well but only temporarily to sulphapyridine. The drug is given in moderate doses for two or three days, then stopped, and repeated after a week's interval.

Both acute and chronic cases of lupus erythematosus may respond to sulphonamides by mouth, but results are very uncertain.

In seborrhœic dermatitis and infective eczemoid dermatitis, successful results have been obtained, particularly where streptococcal organisms are present.

Wounds.—In the concentration of sulphonamides ordinarily achieved in wounds, phagocytosis is not adversely affected, fibroblasts continue to grow at the normal rate and muscular tissue is not injuriously affected. Even with very high concentrations, the growth of fibroblasts, macrophages and epithelial cells is only inhibited and is resumed when the drug concentration is lowered. Brain tissue is, however, irritated by local application of sulphonamides, especially with sulphathiazole enables the maintenance of adequate concentrations in the affected less irritant and sulphanilamide the least irritant, and the use of these two drugs in small amounts is usually harmless. The sulphonamides penetrate from the cavity of the wound into the crevices and deeper tissues. Dead tissues are also penetrated, but to a small extent. Absorption is more rapid from a recent wound than from an oldstanding wound. Absorption is rapid from serous cavities and peritoneal surfaces, but very slight from bony cavities.

In practice, equal parts of sulphanilamide and sulphathiazole are used because its solubility ensures rapid penetration, and the slow absorption of sulphathiazole enables the maintenance of adequate concentrations in the affected tissues. Sulphadiazine possesses low solubility, but has been very sucessfully used in experimental staphylococcal infections of wounds, and should be given a trial in suitable cases. Marfanil, and a new compound "V 187" (p-methylsulphonylbenzamidine), are not inhibited by p-aminobenzoic acid or by pus, and are specially potent against the clostridia causing gas gangrene. They may, therefore, be employed where there is a risk of gas gangrene.

In the treatment of fresh wounds, to prevent infection, careful preliminary preparation of the wound is of the greatest importance and should be carried out as early as possible. All necrotic tissue should be removed, and the wound irrigated with saline solution. A mixture of equal parts of sulphanilamide and sulphathiazole in powder in microcrystalline form, is then blown over the raw surface in a thin film with a powder blower, and the wound closed without drainage if possible. It is important that the powder should be sterilized before application to avoid risk of introducing spore-bearing anærobes into the wound. If local anæsthesa has to be used, local anæsthetics such as nupercaine or stovaine which do not inhibit the activity of sulphonamides, should be employed. More than 15 gm of the sulphonamides should not be used at one time in a recent wound to avoid the risk of reaching dangerous blood levels. The application of the powder is repeated each time the wound is dressed. If the risk of sepsis is great, as in severe lacerations of the muscles or in compound fractures, oral therapy is given in addition to the local applications.

In the field or at first-aid posts, or where immediate surgical attention to the wound is not possible, it is best to apply 5 to 15 gm of sulphanilamide powder at once to the wound, or if sterile powder is not handy, 4 gm of sulphadiazine may be given by mouth.

In the treatment of suppurating wounds, the local application of sulphonamides is not of any particular value, though Marfanil has been widely used in Germany with success, as its action is not inhibited by the presence of pus, and it is also more potent against *Cl. welchii* than the other sulphonamides. Sulphona-

mides administered by mouth are of value in cellulitis, lymphangitis or if bacteræmia complicates the wound or when osteomyelitis is also present. Sulphathiazole, sulphadiazine or sulphamezathine should then be given in moderate to high dosage according to the severity of the case.

In the treatment of compound fractures, very good results have been obtained following the local instillation of sulphonamide powder and primary closure of the wound. Secondary infection does not generally occur, union is more rapid, and the incidence of osteomyelitis is greatly reduced. Good results have also been obtained even in old compound fractures with active infection and sinuses.

Burns.—For first-aid treatment pending removal to a hospital, a 3 per cent water soluble sulphanilamide cream should be applied to the affected area, without attempting to clean the wound or removing blisters. After the patient has been conveyed to a hospital, shock is treated and the area is cleaned and blisters removed. A 10 per cent sulphanilamide cream is then applied to all the burnt areas, covered over with gauze or oil silk and left in position for 6 to 10 days after which the cream may be re-applied if necessary. Alternatively, burns on the trunk and limbs are coagulated by a tannic acid jelly containing 5 per cent sulphathiazole or sulphadiazine, and the above mentioned sulphanilamide cream applied only to the face, hands and genitals. A 3 per cent solution of sulphadiazine in 8 per cent ethanolamine has also been used as a spray to produce coagulation. Sulphanilamide is also used as dusting powder for wounds. This should, however, be replaced by wet sulphanilamide packs if, after repeated applications, a dried crusted surface is produced.

In extensive burns, not more than 15 gm of sulphonamide should be applied in 24 hours, to avoid the danger of excessive absorption. The blood concentrations should be estimated at frequent intervals, and ample amounts of fluids and alkalis should be given concurrently to avoid toxic symptoms.

(14) Miscellaneous Diseases

In tularaemia, sulphathiazole in doses of 6 gm daily produced some benefit, although the improvement is not dramatic.

Malaria.—The sulphonamide compounds have been shown to possess a very definite antimalarial action on the plasmodia in both monkey and human malaria. P. falciparum infections respond better than P. vivax, and the sulphonamides may be used in moderate dosage if quinine, mepacrine, paludrine or chloroquine are not available. Sulphonamides should not be given in combination with quinine, as this combination has been reported to be toxic. They may, however, be combined with mepacrine. The sulphonamides are slower in action and are less effective than either quinine or mepacrine, and relapse occurs in a very large proportion of the cases. Sulphathiazole, sulphadiazine, sulphadimethylpyrimidine and sulphapyridine are all much more active than sulphanilamide.

Amoebic dysentery.—The sulphonamides possess no significant activity against the *E. histolytica.* In cases of amœbic dysentery which do not respond to the ordinary amœbicidal drugs, good effects have been reported with a combination of penicillin (total 2 million units) and succinylsulphathiazole (20 gm daily to a total of 80 gm) in suppressing the secondary infection.

Filariosis.—The sulphonamides have been found to have a beneficial effect on the secondary streptococcal lymphangitis and cellulitis occurring in this disease. There is no effect on the filaria. Any one of the compounds suitable for use against streptococcal infections may be used in moderate dosage.

PART IV

CHAPTER III

ANTIBIOTICS

Penicillin

HISTORICAL AND GENERAL—CHEMISTRY: PHYSICAL AND CHEMICAL PROPERTIES OF PENICILLIN: SALTS OF PENICILLIN, INACTIVATION OF PENICILLIN—MANUFACTURE OF PENICILLIN: PROCESS OF MANUFACTURE, SURFACE CULTURE, SUBMERGED CULTURE, BRAN CULTURE, PROPERTIES—PHARMACOLOGICAL ACTION AND TOXIC EFFECTS: ABSORPTION AND EXCRETION; TOXIC EFFECTS—ANTIBACTERIAL ACTIVITY IN VITRO: SELECTIVE ACTION, INHIBITORS—MODE OF ACTION: BACTERIOSTATIC AND BACTERICIDAL, CHANGES IN BACTERIA AND TISSUE CELLS, RESISTANCE TO PENICILLIN (PERSISTERS)—ANTIBACTERIAL ACTIVITY IN VIVO: ANIMAL EXPERIMENTS; SENSITIVITY OF ORGANISMS: METHODS OF DETERMINATION, LIST OF SENSITIVE AND INSENSITIVE ORGANISMS—PENICILLIN IN THERAPEUTICS—MODE OF ADMINISTRATION: LOCAL, SUBCUTANEOUS ROUTE, INTRAMUSCULAR ROUTE, INTRAVENOUS ROUTE, OTHER ROUTES—EFFECTIVENESS AGAINST INFECTIONS: INFECTIONS OF BLOOD STREAM AND HEART: SEPTICAEMIA AND PYAEMIA, SUB-ACUTE AND ACUTE BACTERIAL ENDOCARDITIS AND OTHER CONDITIONS; INFECTIONS OF THE CENTRAL NERVOUS SYSTEM AND SPECIAL ORGANS: MENINGITIS, WOUNDS OF BRAIN AND BRAIN ABSCESS, INFECTIONS OF THE EYE, EAR, NOSE AND THROAT; INFECTIONS OF RESPIRATORY TRACT; INFECTIONS OF ALIMENTARY TRACT; BONE AND JOINT INFECTIONS; INEFCTIONS OF THE SKIN; GENITO-URINARY INFECTIONS; VENEREAL INFECTIONS; SYPHILIS AND OTHER SPIROCHAETAL INFECTIONS; MISCELLANEOUS INFECTIONS; PENICILLIN IN VETERINARY PRACTICE; PROPHYLACTIC USES; CONDITIONS SUSCEPTIBLE AND INSUSCEPTIBLE TO PENICILLIN—METHODS OF ASSAY AND STANDARDIZATION—PHARMACEUTICAL ASPECTS OF PENICILLIN—PENICILLIN AND SULPHONAMIDES.

Other Antibiotics

STREPTOMYCIN—NEOMYCIN—AUREOMYCIN—CHLOROMYCETIN— POLYMYXIN— TYROTHRICIN (GRAMICIDIN & TYROCIDIN)—GRAMICIDIN—STREPTOTHRICIN—MISCELLANEOUS ANTIBIOTICS.

*PENICILLIN

Oxford Workers.

1. Historical and General

Pasteur and his colleagues (1877) found that certain air-borne organisms inhibited the growth of anthrax bacillus and were the first to make the suggestion that the phenomenon of antibiosis could possibly be used in the treatment of certain infections. This inhibition has now been shown to be due to production of certain substances which have definite chemical and biologic properties. As early as 1899, certain products of *B. pyocyaneus* were found useful in the treatment of anthrax and diphtheria, and in 1929 Fleming reported the discovery of penicillin. This observer found that around a large colony of contaminating mould, the colonies of staphylococci became transparent and underwent lysis. This mould belonged to the genus penicillium and he named the antibacterial substance "penicillin". The mould was later identified as *Penicillium notatum* a species allied to *P. Chryogenum* (*Thom.*). It was shown later that broth filtrate containing penicillin possessed remarkable selective antibacterial properties. It produced inhibition of growth of a variety of gram-positive pathogenic organisms but was relatively ineffective against gram-negative organisms such as *Escherichia coli, H. influenzae,* etc. Fleming further observed that penicillin filtrate was relatively nontoxic for animals or cellular elements, including leukocytes, and it was non-irritant and non-toxic to the conjunctiva.

[*In preparation of this Section I gratefully acknowledge the help I have received from "Penicillin and its practical application" by Sir Alexander Fleming, Butterworth & Co. Ltd., London, 1946 and "Penicillin, its properties, uses and preparations, the Pharmaceutical Press, London, 1946.]

Chain and Florey (1938) made an intensive survey of such naturally produced antibacterial substances from chemical and biological standpoint and one of the first to be taken up was penicillin. They described methods of purification of penicillin and its bacteriostatic properties in vitro and also its chemotherapeutic action in animals mostly using the sodium salt of penicillin. One of the chief difficulties was that penicillin was very unstable and sufficient quantities could not be produced for experimental purposes. In 1941, they prepared fairly pure penicillin and found that it could retain its potency for months if kept in a refrigerator at 4°C. Heating momentarily to 100°C had little effect but after boiling for one hour its potency was lost entirely; heating for half an hour at 55°C did not reduce its potency, but autoclaving destroyed it entirely. When administered to the patients, penicillin was recovered from the urine of patients showing that it passed through the human body. Florey then went to the United States of America and carried on his investigations in collaboration with the workers there. The work was then taken up by the American investigators in Pennsylvania and at the Mayo Clinic. Clinical trials on an extensive scale were taken up. On account of the exigencies of the World War, II, intensive work was done on penicillin and stable salts were produced for clinical use. In 1942 the first patient suffering from meningococcal meningitis was successfully treated with intramuscular and intrathecal injections of penicillin and soon its efficacy in many infections was established.

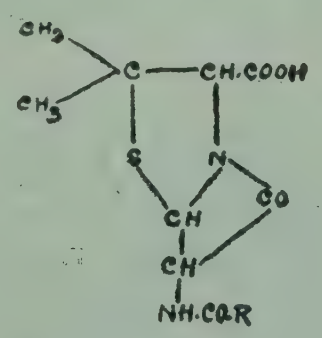
It was first believed that there was only one penicillin but by the method of partition chromatography it was shown that there were at least two if not more. By using different strains of *P. notatum* and by addition of different organic compounds to the medium on which growth is effected, a number of different substances with activity of penicillin have been produced. There is therefore a class of penicillins having a similar general structure.

2. Chemistry

Chemistry.

In Great Britain, the penicillins so far isolated are named Penicillin I, II, III and IV and in U.S.A. the corresponding penicillins are named F, G, X and K. According to Dale all these penicillins have specific biological action in a high, though not quite identical degree, and that there are proportional differences still to be explored in their efficiencies against different organisms. Penicillin II (G) is predominant in most preparations now in common use.

The different penicillins so far examined have the same general structure and vary only in the nature of their side chain R. The molecular formula is C_9 E_{11} C_4 N_2 SR. The graphic formula has a B-lactam ring. R varies with different penicillins.



Pure penicillin is a rarity, but practically pure crystalline sodium and calcium salts are available which are very potent (1600 units per mgm). These are stable and their toxicity is negligible. They can be used in sensitive individuals and also for intra-thecal administration and instilling into the conjunctiva.

The penicillins are monocarboxylic acids and these are unstable in solution. For clinical purposes the sodium salt, which is freely soluble in water and gives a stable neutral solution, is generally used at present. The carboxyl group can be esterified and several esters have been prepared which are inactive in vitro but in vivo they produce the usual action of penicillin.

Penicillin breaks up completely by hydrolysis with dilute acids with the production of amino-acid $\beta\beta$-dimethylcysteine known as penicillamine, corresponding to d or unnatural series of amino-acids (those isolated from normal proteins belong to g series). Co_2 is also produced during hydrolysis at the same time acylated aminoacetaldehyde. The instability of penicillin in alkaline or alcoholic solution is due to opening up of β lactum ring with the formation of penicilloic acid derivatives. Enzymatic breakdown of penicillin by penicillinase of bacterial origin leads to production of acid.

The structure of the penicillins is thus relatively simple, consisting of two amino-acids, dimethylcysteine and an acylated series, the alcoholic group of which is oxidised to the aldehyde. The peptide structure is incorporated in B lactam ring. There appears to be little hope of commercial synthesis of penicillins at present. There is possibility that by modifying the hydroxybenzyl group in penicillin-III additional penicillins may be produced which may be even better than the natural compounds.

(1) Physical and Chemical properties of penicillin

The commercial material produced in surface culture plants is mainly penicillin-II and the same form is obtained from deep culture plants of the mould used and conditions under which it is grown. Penicillin produced by some American firms contains penicillin-IV and sodium salt of Penicillin-II in pure state.

(1) *Salts of penicillin.*—Free penicillin exists as a strongly dibasic organic acid and forms various salts and esters. When exposed to air and when heated, both penicillin and its salts rapidly lose their activity. Contamination with air bacteria destroys penicillin. Free penicillin is very soluble in ether, alcohol, acetone, ethyl acetate, amyl acetate, etc. It is not so soluble in benzene, chloroform and carbon tetrachloride. It is soluble in water to the extent of 5 mgm per ccm. It is very unstable in contact with dilute acids, alkalies, primary alcohols, oxidizing agents and heavy metals. It is also unstable below $pH_{5.6}$ and above pH_7.

Sodium, Calcium and other salts. Sodium and calcium salts of penicillin are largely used. When purified these salts occur in crystalline form, the crystals of sodium salt look somewhat like fence pickets, one end being pointed and other straight. The calcium salt contains about 5.6 per cent of calcium. The calcium salt unlike the sodium salt is not hygroscopic and it can be stored in the dry state in sealed ampoules for as long as six months at room temperature without any apparent loss of activity. Barium, potassium, magnesium, ammonium and silver salts of penicillin have been prepared. Barium salt is stable for an indefinite period in dry state and is a non-hygroscopic dry powder.

Methyl, ethyl, and benzyl esters of penicillin have been prepared. These esters are insoluble in neutral or slightly alkaline buffers. The esters are soluble in benzene; the aliphatic esters are less active in vitro than penicillin, but have considerable activity in vivo, probably due to hydrolysis and consequent liberation of penicillin. It is possible that they may not be destroyed by gastric juice and possibly some of the higher esters could be administered by mouth.

(2) Inactivation of penicillin

Penicillin is inactivated by contact with various heavy metals (copper, lead, zinc, etc.) in a manner which is not understood. It is inactivated by such bases as ammonia, aniline or quinine in the ionised state.

Ethyl alcohol in high concentrations destroys penicillin and, therefore, should be rigorously excluded. There is, however, no objection to its use to sterilise rubber caps of penicillin vials. Inactivation is due to splitting up of β-lactum ring and formation of ester of penicillinic acid. Glycerine has an unfavourable effect on the keeping properties of aqueous solutions of penicillin. The amino-acid cysteine rapidly inactivates penicillin and is used for freeing susceptible organisms from it. Phenolic substances (phenol, phenoxetol, etc), chlorobutol (0.5 per cent) rapidly increase the rate of deterioration. Adrenaline which contains chlorobutol as preservative also does the same. Oxidising agents such as potassium permangnate, hydrogen peroxide should be avoided. Thiomersalate rapidly causes inactivation.

Penicillinase, an enzyme produced by certain bacteria, rapidly inactivates penicillin. Its temperature of maximum activity is 37°C and the optimum pH is 6.5 to 8. Impurities in penicillin retard its action. It is curious that staphylococci that become resistant to penicillin in vivo during treatment of infection with penicillin develop ability to form penicillinase but not those which become resistant in vitro. It may be mentioned that the naturally resistant species of bacteria owe their resistance to their possession of *penicillinase*, the enzyme which destroys penicillin.

Traces of substances containing sulphydryl groups such as sodium thioglycolate increase its activity in low concentrations (0.01 per cent) but retard it in higher concentrations (0.5 per cent). An enzyme resembling penicillinase named clarase has been obtained from the mould aspergillus flavus; this enzyme is preferable to penicillinase for destroying activity of penicillin.

Some samples of rubber tubing both natural and synthetic are liable to inactivate penicillin and repeated sterilisation of rubber increases the rate of inactivation.

Penicillin does not reduce Fehling's solution but the colour is changed to green. Slight change may, therefore, occur in the colour of urine of patients, taking penicillin when tested with Fehling's solution or oxidizing agents such as hydrogen peroxide and potassium permanganate, which rapidly oxidize penicillin thus producing loss of its activity. Penicillin is less sensitive to reducing agents and the reduced material retains its activity.

3. Manufacture of Penicillin

(1) The process of manufacture of penicillin may be discussed under the following heads.

Preparation of medium.—All media now contain "Corn Steep liquor" (obtained by steeping maize) which has a substance which stimulates the growth of penicillin. The media are generally based on CZAPEK-DOX medium and the following is a modification in use that has been found to be satisfactory:—

Sodium nitrate ($NaNO_3$) 3.00 gm, potassium acid phosphate (KH_2PO_4) 1.00 gm, potassium chloride (KCL) 0.50 gm. magnesium sulphate ($MgSO_4.7H_2O$) 0.5 gm, ferrous sulphate ($FeSO_4.7H_2O$) 0.01 gm, glucose 40.0 gm, distilled water, q.s. 1,000 ccm. Different investigators have produced different modifications of this medium and some have substituted brown sugar for glucose. It was further demonstrated that the presence of zinc considerably promotes the growth of the mould and increases the yield of penicillin, the optimal concentration of zinc in this connection being 1 to 3 mgm of zinc sulphate per litre. Zinc probably acts as a catalyst and promotes the oxidation and utilization of glucose by the mould thus preventing accumulation of gluconic acid which produces a fall of pH of the medium. It has also been shown that ferrous sulphate and potassium phosphate could be reduced. The addition of monohydrogen phosphate and dihydrogen phosphate for purposes of buffering the medium is, however, desirable.

Sterilization and cooling of medium.—Sterilizers of special designs are used and for mass cooling of penicillin flasks and special coolers have been designed.

Preparation of spore suspension and inoculation.—Great care has to be taken to ensure the purity of the parent culture from which subcultures are prepared for manufacturing units. Suspensions of spore are made with a suitable wetting medium to secure uniform distribution and a simple automatic pipette delivering a constant volume is used. The operation is carried out inside a large incubator in some of the large factories with a spray gun. Incubation time and temperature depend on each other and on the particular strain used.

Deep Fermentation and Harvesting.—Here sterilizing, cooling, inoculation and incubation are all carried out in the same vessel of which batteries are assembled.

After incubation, the liquid under the mould mycelia is removed from the mould into tanks and broken up mycelia are removed by filration. Great care is taken that bacterial contamination does not occur.

Concentration and drying.—By suitable adjustment of pH and choice of solvents, and sometime in combination with charcoal adsorption stage, a concentration many times the original metabolite solution at harvesting is reached. The idea is to produce a concentrate containing about ten per cent of solids suitable for drying. The yellow colour of penicillin is due to pigment chrysogen which is a contaminant in the finished product.

Drying is done by spray and freeze drying methods, the latter needs a special plant which entails considerable expense. This method is similar to that used for drying human plasma in bulk. The dried powder is stored and weighed under aseptic conditions. The best method of freeze drying is that which involves filling of intended vials with the 10 per cent solution and drying it there.

During all stages in the process of manufacture very strict supervision by experts is essential. Three methods are generally employed for manufacture of penicillin:—

(2) *Surface culture method.*—Most of the penicillin used in the early studies was produced by the surface culture methods in which the spores of the mould are seeded on the surface of the culture medium which is 1.5 to 2 cm in depth. Usually 50 ccm of the medium are used in a 200 ccm Erlenmeyer flask, but bottles or even large pans may be used. The medium is seeded with spores and the mould is found to grow well at temperatures between 22°C and 25°C; it fails to grow at 37°C. Usually 24 hours after seeding, growth begins to appear, and on the 3rd day the whole of the surface is covered with a white growth. On the fifth day the growth becomes green and somewhat rigid. In the surface cultures the pH of the medium, which starts at 3.5 to 4.5, remains the same for three to four days, but as the growth of mould becomes more profuse a rise in pH occurs. This is accompanied by rapid development of antibacterial property which reaches its maximum when the pH approaches the neutral point. In order to preserve the antibacterial titre, the mould should be filtered off and the pH of the medium adjusted to 6.5 to 7. The crude broth filtrate is separated and may be kept at icebox temperature without losing its activity for 7 weeks provided the pH is adjusted to 6.5 to 7. Frozen crude penicillin broth can retain its activity for several months.

Advantages of surface culture.

The advantages of this method are that the yield is fairly high and the equipment necessary is simple. A great advantage of growing penicillin by this method is that the individual units are small and if contamination occurs it does not destroy the whole plant. Bacterial contamination is a very serious trouble. Gram-negative bacteria such as *E. coli, B. subtilis,* etc., produce enzymes which rapidly destroy the penicillin present. It is, therefore, very essential that all contamination should be rigidly excluded.

(3) *Production from submerged cultures.*—This method was largely studied in the United States Department of Agriculture and also by certain commercial concerns. To produce submerged growth. it is essential to effect aeration by constant agitation of the culture. This is done by shaking the flasks and by designed aerators; rotary drum fermenters equipped with aerators and agitators are also used. Under these circumstances the mould grows as small pellets. The advantage of submerged methods is that enormous time and labour are saved. The disadvantage is that when contamination occurs penicillin produced in the whole batch is lost.

(4) *Production of Penicillin on Bran.*—This method consists in growing a suitable strain of *P. notatum,* on moist bran which is thinly spread in suitable trays, the bran being first sterilized and then inoculated with the culture of the mould. By this method it is possible to produce as much as 200 to 400 units of penicillin per gram of dried bran after it has been allowed to grow for several days. The penicillin is extracted by the use of suitable solvents. The difficulties of this method are that bran is a very poor conductor of heat and is difficult to sterilize and thus to exclude contamination. The other difficulty is that considerable heat is produced during fermentation which is not easy to dissipate.

Small scale preparations.

For small scale production 30 gm of wheat bran are placed in conical 750 ccm flasks the bran being mixed well. The flasks then plugged and autoclaved at 15 lbs of pressure for one hour. The sterilized flasks are then inoculated with 1 ccm of suspension of spores of *P. notatum;* the inoculated material is shaken and incubated at 24°C for two days. By this method as much as 150 units of penicillin per gram may be produced (Srinivasa Rao).

(5) *Properties of penicillin.*—The stability of penicillin depends on the presence of moisture and if this is rigidly excluded, penicillin will not lose its activity for a year even without storage in a refrigerator. The U.S.A. Food and Drugs Administration require that the moisture content of crystalline sodium penicillin shall not exceed 1.0 per cent and that it shall not lose more than 25 per cent of its potency when stored at 100°C for six days. The label need not bear any special instruction with regard to storage conditions.

The sodium salt is highly hygroscopic and the calcium salt least hygroscopic. In this connection it should be remembered that rubber plugs which seal the vials are not entirely impervious to moisture and even with the aluminium seal and dust cap, appreciable amounts of moisture may diffuse into the vial and deterioration may begin. Investigations in this connection show that when stored below 10°C and protected from moisture, penicillin which has been adequately dried will remain active for a year. At 37°C dried powder retains its full potency for 60 days.

Solutions of the barium salt have maximum stability at pH 5.5 to 7.5, at 2°C for several weeks; boiling for 30 minutes renders it inert. Sodium penicillin-II in solution has a maximum stability at pH 6 and will lose its original activity by 50 per cent in 14 days at 24°C. With calcium and sodium salts the rate of deterioration is less in dilute solutions than in more concentrated solutions. Solutions kept at room temperature (22°C) retain full activity for 10-12 days and at 37°C for 4 days.

The effect of temperature on deterioration is less on more purified penicillin than on less purified. There is an initial lag in deterioration but once it begins the rate progresses rapidly, probably owing to increasing activity of solution as a result of decomposition.

Increase in rate of decomposition with rise of temperature is rapid. The solutions should , therefore, be stored in a refrigerator or on ice. In cold storage, solutions will retain their activity for a week at best.

No information is available as yet with regard to the properties of four varieties of penicillins so far identified. It is probable that at a reaction near neutrality the activity is appreciably longer

4. Pharmacological Action and Toxic Effects

(1) Absorption and excretion

Subcutaneous, intramuscular and intravenous routes.

(i) *Absorption.*—Penicillin is readily absorbed and diffuses well into most of the body tissues and fluids. When given by the intravenous, intramuscular or subcutaneous routes penicillin is readily absorbed and its presence can be determined by various methods. Little information is available as to what constitutes an optimum or even an adequate blood level effective in the treatment of various diseases. Practical experience has shown that penicillin therapy often produces remarkable effects when penicillin blood level is below the stated effective figures for much of the time as is the case when intermittent injections are being given.

Penicillin disappears from the blood quickly after a single intravenous injection although it attains a high concentration for a short time. Given by the subcutaneous route, the concentration in the blood is well maintained but detectable amounts are present here for longer periods than after a single intravenous injection. After a single intravenous injection the concentration in the blood reaches its highest point and then rapidly falls, the peak of concentration depending on the quantities of penicillin administered.

The amounts of penicillin sufficient to have inhibitory effect on micro-organisms are no longer present three hours after a single injection. After intramuscular injection of 50,000 i.u. of penicillin in a single dose the blood obtained one hour after injection did not contain more than one unit of penicillin per ccm. Three hours after injection no penicillin activity could be detected in the blood. When continuous intravenous drip method is used the activity of penicillin is more or less maintained at a constant level.

Oral and rectal administration. Little or no absorption of penicillin occurs if it is given by the oral route under normal conditions. This is due to the fact that it is partly inactivated by the hydrochloric acid of the gastric juice and partly by the enzyme penicillinase which is produced by the intestinal flora. In patients suffering from pernicious anæmia where there is no acid in the gastric juice, therapeutically active concentrations of penicillin can be obtained in the serum of patients after oral administration. In achlorhydria, therefore, penicillin could be effectively given by the oral route. In experimental animals, effective concentrations of penicillin in the blood can be obtained by giving large quantities of bicarbonate of soda with penicillin. The absorption, however, in this way is very erratic. Penicillin can be absorbed from the duodenum and inhibitory concentrations in the blood could be obtained and steadily and persistently maintained by administering penicillin through the duodenal tube. Recent work, using larger doses such as 50,000 to 100,000 units, has shown that therapeutically effective serum levels may be obtained and maintained by oral administration.

Potassium salts give higher and more sustained concentrations than the sodium or aluminium salts. It is customary to administer such antacids as aluminium hydroxide, magnesium trisilicate etc., along with the penicillin salt, to counter the effect of the gastric juice.

Serous cavities.

When given per rectum little absorption of penicillin occurs on account of its inactivation by the faecal organisms. Penicillin can be absorbed and detected in the blood after injection into inflamed cavities such as pleura, peritoneum, joint cavities or the spinal canal. Absorption, however, is not rapid and it remains at the site of injection for as long as 24 hours. It is also absorbed into the blood after its introduction into the bronchii by means of sprays, etc.

Rapidity of absorption.—The case and rapidity with which penicillin is absorbed are responsible for the difficulty in maintaining constant concentrations of this substance in the body tissues. It is, therefore, necessary to use frequent injections or continuous infusions. Efforts have been successfully made to delay the absorption of penicillin from the subcutaneous and intramuscular routes by suspending it in beeswax and oil mixtures (pea-nut oil or sesame oil) Recently, a compound of penicillin with procaine in a suitable oil base with the addition of aluminium stearate has proved very successful in delaying absorption adequately, so that a single dose of 300,000 units maintains effective therapeutic levels for 24 to 48 hours.

Intrathecal injections.

(ii) *Diffusion of penicillin into tissues.*—It has been shown that red blood corpuscles absorb very little penicillin (10 per cent of the amount present in the plasma). No penicillin activity can be demonstrated in cerebrospinal fluid of cats following intravenous injection and the same is the case with man even after intravenous drip method. When penicillin is injected by the intrathecal route, it remains in the spinal fluid for at least 24 hours; 48 hours later, no penicillin activity can be detected. In infections of the crebrospinal system, penicillin should be given by this route at least once in 24 hours to maintain adequate concentrations. When the meninges are actually inflamed and also in meningococcal meningitis, penicillin is believed to reach the spinal fluid after intravenous injections. These findings have not been fully confirmed.

No penicillin activity can be detected in tears after systemic administration of therapeutic doses. The concentration of penicillin in the vitreous humor and cornea is usually small. For treatment of infections of the eye, therefore, penicillin should be applied locally as well as systemically. Small penicillin activity can be demonstrated in the saliva after its systemic administration.

Peritoneal and pleural cavities.

Penicillin is not detected in the pancreatic juice after systemic administration though pancreatic tissues may contain some after intramuscular injection of large doses. Penicillin diffuses rapidly into the peritoneal cavity and can be detected within five minutes after an intramuscular injection; the concentration rises even higher relatively than in the blood.

Penicillin remains in the pleural cavity for a considerable time after its direct introduction. Intra-pleural injection of penicillin is indicated in the treatment of infections of the pleura.

Joints.

After systemic administration of penicillin, antibacterial amounts reach the fluid of inflamed and normal joints, the concentration being the same as that occuring in the blood.

Mother and foetus.

If a large amount of penicillin is given by the intravenous drip method to a woman in labour, the blood obtained from the umbilical cord after delivery and also from the mother will contain penicillin. Penicillin, therefore, diffuses through the placenta and becomes available to the foetus.

Penicillin reaches all the tissues of the body with the exception of the central nervous system and the bone marrow, at least in some concentration after systemic administration. Even after massive injections no penicillin could be detected in these tissues. *Nervous tissue and bone marrow.*

(iii) *Excretion.*—Penicillin is readily excreted by the normal kidneys and can be recovered from the urine of all patients who have received it; 50 to 60 per cent of penicillin given is fairly rapidly excreted by this route. After a single intravenous injection the urinary excretion of penicillin is completed within the first hour of its administration. Coliform bacteria in the urine produce a rapid loss of penicillin activity especially if the urine is kept at room temperature.

If renal disease or renal failure or urinary suppression is present excretion is delayed and high concentrations of penicillin can be maintained in the blood. Attempts have been made to maintain the concentration of penicillin in the blood by depressing the kidney function by addition of diodrast to the material for intravenous injection. Administration of para-aminohippuric acid to experimental animals to which penicillin was being given, reduced considerably the renal excretion and enabled maintenance of higher level in the plasma. *Renal disease.*

Penicillin is excreted in the bile of animals after intravenous injections Appreciable amounts of penicillin reach the liver after systemic administration. *Liver and Bile.*

(2) Toxic effects

The toxicity of penicillin to the mammals is very low in contrast to its toxic effects on bacteria. Fleming, by rapid successive intravenous injections, raised its concentration in blood 1,000 times greater than that required for therapeutic purposes without untoward effects. In ambulatory cases of syphilis, enormous quantities have been given without harm. The purer the penicillin the less its toxicity, in fact it has been claimed that penicillin of good quality has no toxicity.

The toxicity of penicillin salts of great purity is due entirely to the cation (Na, Ca, Li, etc.). The calcium salt is six times more toxic than sodium salt but this would only come into evidence in man of 60 kilo bodyweight after 10 millions units are given.

(i) *Toxicity to animals.*—As purification of penicillin has progressed, the lethal dose for mice has increased. The lethal dose of products manufactured by various manufacturers varies widely. It has been shown that guinea pigs are particularly sensitive to the toxic action of penicillin. It is possible that penicillin destroys the normal bacterial intestinal flora in these animals, which is predominently gram-positive, and in this way interferes with the metabolism of certain substances as are essential for the health of this animal.

Toxicity in man.—Toxic effects are few and of a minor nature and no damaging effects on the liver, lungs, kidneys, bone marrow, nervous tissue, etc.. have been observed even with very massive dosage.

The most sensitive tissue to inferior quality of penicillin is the nervous system and meningeal irritation (with cerebrospinal fluid pleocytosis) occurs after intrathecal injections. Excessive doses may damage the brain and spinal cord and convulsions have occurred after injection into the lateral ventricles.

Thrombophlebitis may occur after continuous intravenous transfusion needing a frequent change of vein; certain batches may cause fever, urticaria, and local reactions but these are due to impurities, as also are such reactions as meningeal irritation after intrathecal use. Sensitisation phenomena such as urticaria and dermatitis following local application rarely occur.

(ii) *Toxic symptoms in man*

Local and general reactions.—It has been shown that penicillin sometimes produces toxic reactions both in animals and man:—

(1) Pain of a stinging nature at the site of injection usually occurs and brands of penicillin vary in their tendency to produce this pain. It is probably not due to penicillin but to associated substances. The pain after injection passes off in few seconds. A dull pain may remain at the site for half an hour or so and the part may remain tender for 24 hours. This may be sufficient to worry some patients and make them dread the treatment. To minimise the pain, the drug should be dissolved in very small quantities of saline solution to prevent larger quantities producing mechanical distention of muscle fibres and consequent irritation.

(2) Venous irritation at the site of injection frequently occurs (in 5 to 10 per cent) specially if penicillin contains impurities. If this occurs the site of injection should be at once changed. Veins of the arm are preferably used for injection because if thrombosis occurs in the veins of the leg, embolism may follow.

Fever and chills.

(3) Fever and chills are due to the presence of pyrogens and improperly sterilised apparatus used for injection. Since pyrogen free penicillin is now available these are not often encountered. Any bacterial contamination with water organisms may produce chills. Fever and chills occur in 5 to 10 per cent of cases.

Cutaneous reactions.

(4) Urticaria has been known to occur following local or systemic use of penicillin. It may occur later in the course of treatment even after penicillin has been discontinued for a week or more. Urticaria, when it occurs, can be controlled with adrenaline and calcium.

(5) Incidence of delayed serum reactions after penicillin is low being no greater than 1 in 1,500 to 1 in 2,000. The features observed are delay in appearance, severe malaise, mild fever, severe spreading urticaria, arthralgia and effusion into joints, exfoliative dermatitis of palms of hands and self-limited course of 7 to 10 days. Everything points to its being sensitization to penicillin fraction in susceptible individuals.

Subcutaneous type of reactions such as pruritis, urticaria toxic erythemas, vesicular and bullous dermatitis of hands and feet, herpes simplex, pityriasis, roseoform dermatitis, and varying degrees of contact dermatitis and even exfoliative dermatitis have been observed. Contact dermatitis may occur after local application of ointments of penicillin. Patch test with penicillin is often positive in such patients. Positive intracutaneous and scratch test, however, do not necessarily show sensitiveness to penicillin.

Abdominal pains.—Patients receiving penicillin sometimes get colicky pains which may be mistaken for appendicitis. Besides the above, mild headaches, flushing of the face and muscular pains have been met with after administration of penicillin. Diarrhaea, nausea and vomiting may occur.

Uterine reactions.—It has been shown that penicillin may stimulate bleeding and uterine contractions both in the pregnant and non-pregnant uterus. A number of cases of threatened abortion have been recently reported in patients undergoing penicillin therapy for syphilis, and it has been observed that menstruation may be initiated during the first 24 hour period of penicillin-therapy.

Deficiency of nicotinamide may occur after oral administration and produce black tongue.

(iii) *Toxic reactions of penicillin*

These are grouped by Morginson (1946) into:—

(a) *Direct toxic and primary irritant.*—Penicillin has very little direct toxic action on tissues and in experimental animals, 400,000 units produce no injury to kidneys, liver, brain, bone-marrow, hæmopoietic system, etc. The universal direct toxic action of penicillin depends on impurities contained in the corn-mash or steep, chemicals used to remove pyrogens, or other products. For practical purposes the possibilities of penicillin as a direct toxic agent are negligible except perhaps with intrathecal use.

(b) *Antigen.*—Commercial penicillin and crystalline penicillin possess definite antigenic properties which may be excited by impurities encountered in commercial specimens, pyrogens and chemicals used to remove this and crystalline penicillin. There is no corelation between colour of penicillin and its toxicity. Reactions produced by antigen properties are erythema, urticaria, angioneurotic œdema, dyspnœa, dizziness, vertigo, etc. The sensitivity may be manifested by immediate reactions and delayed reactions which may not be noticed until several weeks after injection. Allergic symptoms following penicillin are usually mild, of low incidence and transient.

(c) *Therapeutic shock or Herxheimer reaction occurs* after the use of penicillin in the treatment of syphilis. Cutaneous reactions occur after 8-12 hours or may be delayed for days. Systemic reactions are malaise, chill and fever 103°F. Symptoms subside within 24 to 48 hours. Herxheimer reaction can be avoided by early reduced dosage of penicillin; it is particularly undesirable in debilitated toxic and weak individuals, and in patients with cardiovascular syphilis and syphilis of nervous system.

(d) *Indirect healing action.*—On pathological processes producing "therapeutic paradox" of Stokes may occur on account of deforming sclerosis in such arteries as the coronaries which occurs so rapidly that compensation does not take place. Rapid healing fibrosis may occur in the liver producing obstruction of portal circulation.

(e) *Reaction following intrathecal use.*—Penicillin is believed to have direct irritant actions on the central system and this property is said to be inherent in penicillin rather than in impurities. It has been shown that seven commercial penicillins given intracisternally and intraventricularly produced convulsions and death due to vascular collapse in mice, cats, dogs and monkeys. Intracortical injection produced convulsion in man. Convulsions occur with high dosage only, and these should not detract from its clinical intrathecal use when indicated. Such symptoms are headache, nausea, listlessness, cyanosis, weak and thready pulse, stiff neck, convulsions and coma. Important factors in producing toxic reactions are:—(*i*) concentration of penicillin in solution, (*ii*) slow diffusion of spinal fluid, (*iii*) size of individual doses, (*iv*) and frequency of injections.

Penicillin has very low cytotoxicity and even in 1 in 1,000 dilutions the leucocytes of man remain active for at least three hours. In antibacterial amounts it does not interfere with the growth and migration of tissue elements such as lymphocytes and fibroblasts or macrophages. This is quite in contrast to sulphonamide drugs. In fact patients with low leukocyte count and symptoms of agranulocytosis following sulphonamides have improved with penicillin treatment and for this reason it has been used in the treatment of this condition.

All the toxic reactions above mentioned are easily controlled and none of these endanger life.

5. Antibacterial Activity in Vitro

Gram-positive and gram-negative organisms.

(1) *Selective antibacterial action.*—One of the most important properties of penicillin is its selective antibacterial activity. Its action is weak against most gram-negative pathogenic organisms while gram-positive pathogenic organisms are readily susceptible to its action; even some of the important gram-negative organisms are not resistant. Fleming showed that an inhibitory effect occurs against various *staphylococci, Diplococcus pneumoniae, Str. pyogenes, Neiss.*

gonorrhoeae, Neiss. intracellularis and *Corynebacterium diptheriae.* It has some inhibitory action on *B. anthracis.* Penicillin was relatively ineffective against colon-typhoid group, *B. pyocyaneus (Pseudomonas aeruginosa, Proteus vulgaris* and *Vibrio comma,* Enterococci, *Klebseilla pneumoniae,* (Friedlander's bacillus) and *Hemophilus influenzae* were also insensitive. On account of these properties, penicillin is used to isolate insensitive bacteria from a large number of sensitive organisms. It is also used for demonstration of bacterial inhibition and for treatment of infections with sensitive organisms.

A list of organisms sensitive and insensitive to penicillin is given in page (636). It has, however, been shown that certain pathogenic organisms may be insensitive on the basis of studies made in vitro, and yet penicillin has been found to be effective in vivo in the control of infections produced by some of these micro-organisms.

Different strains and numbers of organisms.

Factors influencing antibacterial action.—Some strains of pneumococci and *Str. viridans* tested were less sensitive than others of the same species. These variations are of practical importance, as they are responsible for varying responses to the treatment with penicillin in different cases of sub-acute bacterial endocarditis. On the whole staphylococci appear to be more uniform in their sensitivity than streptococci.

The number of organisms initially inoculated affects the activity of penicillin. The pH of the medium is also of importance in connection with the bacteriostatic action of penicillin and a pH from 7 to 5.5 enhances the bacteriostatic action of penicillin.

(2) *Inhibitors of penicillin.—E. coli* produces an enzyme like substance "penicillinase" which completely inhibits the action of penicillin. This substance is intracellular since it is not found in filtrate of cultures of this organisms. The enzyme which inhibits penicillin is also found in certain organisms which are sensitive to the action of penicillin, and therefore, is not the sole factor which determines antibacterial action of penicillin against those organisms. Bacteria which produce "penicillinase", however, are not likely to be very susceptible to penicillin. The living culture, the culture filtrates and dried preparations of para-colon organisms all destroy penicillin.

6. Mode of Action

(1) *Bacteriostatic and bactericidal action.*—Penicillin can act as a bacteriostatic or as a bactericide, according to conditions present. When these are favourable to bacterial growth, penicillin acts as a powerful bactericide. The bacterial cells enlarge, the chromosomes begin the process of division, but it is not completed which results in the swelling of the cell. This view, however, is not tenable. It is believed that penicillin interferes with some vital functions of the early bacterial development and thus has a bactericidal effect even before actual cell division occurs. It is possibly an inhibitory effect on enzyme action concerned in metabolism of nucleotides.

With regard to reaction with sulphydryl groups the effect of penicillin probably resembles sulphonamides but the manner in which it is achieved is different. Whereas penicillin may react on sulphydryl groups in the bacterial cell, the sulphonamides act by interfering with the absorption of an essential nutrient. The activity of penicillin is not affected by the number of bacteria present, whereas sulphonamides are much less effective if infection is heavy.

In exceedingly small concentrations penicillin stimulates bacterial growth.

Penicillin kills bacteria in most of the cultures containing *Diplococcus pneumoniae, Str. pyogenes* and *Staph. aureus.* The presence of inactivitated serum frequently reduces the activity of penicillin. It has been found that, within certain limits, the rate of killing increases with increasing concentrations of penicillin. Penicillin appears to be most effective when active multiplication of bacteria is taking place.

Considerable time is necessary for penicillin to act on certain bacteria and bacterial inhibition is not complete unless it remains in contact with the organism for sometime, between 4 to 6 hours. If, therefore, penicillin is to be used as a local agent it must remain in contact with the organisms for a considerable time; merely irrigating the surface is not likely to do much good.

Morphologic changes.

(2) *Changes produced in Bacteria and Tumor cells.*—When *Cl. welchii* is brought in contact with penicillin it shows elongation and takes form of unsegmented filaments ten or more times longer than the original bacteria. Similar changes were found in some of the gram-negative bacteria which are resistant to the action of penicillin. Such changes have been recorded in bacteria with certain dye substances when these are allowed to act on colon-typhoid group of bacteria. It would thus appear that morphologic changes produced in bacteria are not limited to penicillin only. It is curious that the organisms which are sensitive to penicillin often do not show this phenomenon. These changes, therefore, do not explain the antibacterial activity of penicillin. Morphologic changes in coccal forms have been observed in the blood of patients receiving penicillin intravenously. Gonococci found in the urethral discharges of patients become swollen and irregular in shape two to three hours after administration of penicillin. Morphologic changes also occur in *Staph. aureus* cultured in penicillin broth.

Certain types of malignant cells may be affected by the action of penicillin, *e.g.*, mice sarcoma cells. When cultures of these malignant cells are damaged by penicillin they are unable to produce tumours in rats by implantation.

(3) *Persisters.*—It is known that complete sterilisation is not usually obtained with penicillin and while 99 per cent of sensitive bacteria may be killed a few remain unaffected. These are called 'persisters' and it has been suggested that this is due to the fact that such organisms are dormant and in a non-dividing phase. Under suitable condition they resume normal division and are then susceptible to penicillin. This may explain difficulty of curing such conditions as bacterial endocarditis.

Penicillin fastness.

Resistance to penicillin.—The position with regard to development of penicillin resistance to micro-organisms is undecided. The Oxford workers found it possible to increase the resistance to penicillin in a strain of *Staph. aureus* 1,000 times by subculturing this organism in the presence of increasing amount of penicillin over a period of 9 weeks. Penicillin fastness, however, is not easily accomplished in case of organisms such as *Diplococcus pneumonia, Str. pyogenes,* etc. As the resistance to penicillin increases, virulence of the organisms declines. When staphylococci are allowed to become resistant to penicillin they are said to become many times more susceptible to the antibacterial action of human blood.

The question of development of resistance to penicillin is of fundamental importance. It is believed that although resistance on the part of the causative organisms may develop among patients who are under treatment, it does not always account for the poor therapeutic results obtained with penicillin in infections with different pathogenic organisms.

7. Antibacterial Activity in Vivo

(1) *Animal experiments.*—Penicillin was first used in the treatment of infections in vivo in 1940, the mouse being used as an experimental animal. Mice can be completely protected by subcutaneous administration of penicillin when these animals have been given 10,000 to a 100,000 lethal doses of *Staph. aureus* intraperitoneally. The treatment was begun immediately after the bacterial inoculation and penicillin was given every three hours night and day for five days. A dose of 4.0 mgm of sulphathiazole per day was required as compared with one mgm of penicillin to afford similar protection. Ulceration of cornea produced in the eye of rabbits by using virulent strains of staphylococci was treated locally with penicillin every hour starting one hour after inoculation and continued for 48 hours. There are definite beneficial effects on the development of these lesions following local application. Beneficial effects of sulphadiazine on such infected eyes were very much weaker than those produced with penicillin. Mortality was considerably reduced when penicillin was injected intrathecally into mice in whom meningitis had been produced with *Staph. aureus.*

Sulphonamides and penicillin.—The effectiveness of penicillin against experimental infections due to *Strept. pyogenes* was also established and in this respect penicillin has a more potent action than sulphonamides. In case of pneumococcal infections there are variations in sensitivity of different strains to penicillin. Development of resistance to sulphonamides does not interfere with the activity of penicillin. Penicillin protects mice even when given six to seven hours after inoculation, which is not the case with sulphonamides.

Gas gangrene.

Anaerobic infections.—Mice infected with lethal doses of *Cl. welchii* were successfully treated with penicillin and the infection was controlled. Whereas sulphadiazine and sulphathiazole would protect approximately half of the animals infected with this organism, penicillin cured 98 per cent of infected animals provided it was given at the same time as infection. If, however, three hours elapsed between inoculation and beginning of treatment, decrease in survival rate resulted. It has been shown that rather larger amounts of penicillin are required in the treatment of anaerobic infections than aerobic ones. Penicillin is effective in infections due to organisms associated with gas gangrene. Experimental work shows that penicillin has no neutralizing effect against toxins produced by these organisms. It is, therefore, advisable to combine penicillin therapy with antitoxin in the treatment of established infections.

It has been shown that *B. anthracis* is fairly sensitive to the action of penicillin. In mice who were given 10,000 lethal doses of *B. anthracis* and 16 hours were allowed to elapse before the commencement of treatment with 1,000 units of penicillin every four hours, more than half of the animals survived.

Until the discovery of penicillin no form of treatment except administration of sera was effective in the treatment of erysipeloid disease in man, sulphonamides being entirely useless. Penicillin is effective in *in vivo* experiments against this disease in animals and these results justify the speculation that it may prove effective in the treatment of this disease in man.

Corynebacterium diphtheriae is sensitive to penicillin. *Mycobacterium tuberculosis* is insensitive and so are protozoal organisms.

Experimental syphilis.

Spirochaetal infections.—It has been shown that penicillin has a definite spirochætostatic action. In rabbits experimentally infected with syphilis, penicillin showed some spirochæticidal activity, and exerted some prophylactic action also; sometime chancres developed late and the lesions were smaller than usual. It

was also shown that the strains of spirochaetes in rabbits which received insufficient amounts of penicillin became more resistant to the action of penicillin than the original strain. The practical significance of these findings is far reaching. Although the action of penicillin against spirochaetes of syphilis in vitro is relatively feeble its action in vivo appears to be powerful.

Sp. recurrentis and leptospira infections.—The blood of mice infected with strains of spirochæte of relapsing fever (*sp. recurrentis*) could be cleared of spirochætes within 24 hours by subcutaneous injection of 250 units of penicillin. Inoculation of the blood from treated animals into fresh mice did not convey the infection. Although *Leptospira icterohæmorrhagiæ* was insensitive to the action of penicillin in vitro yet it is effective *in vivo* and it is likely that penicillin might be of value in the treatment of Weil's disease.

Spirillum minus.—Rat-bite fever is associated with *Spirillum minus* or *Streptobacillus moniliformis.* In the former condition, organic arsenical preparations are of some value but not with the infection of the latter organism although gold preparations have some effect. Penicillin given intravenously 15 minutes after inoculation of the animal with *Spirillum minus* prevented the development of the disease. Penicillin is said to be more effective than neoarsphenamine and produces complete inhibition of growth of *Streptobacillus moniliformis* in vitro in the presence of 0.1 unit of penicillin per ccm and partial inhibition with 0.01 unit per ccm.

Ornithosis and psittacosis.

Virus and Rickettsial infections.—It is well known that no therapeutic agent is of much value in the treatment of infections produced by virus of ornithosis and penicillin failed to completely eliminate the virus from the bodies of the treated mice. Protective effects, however, were obtained, against these usually fatal infections.

Penicillin also exerts an inhibitory action on the multiplication of Rickettsia which produces murine-typhus. The virus of lymphogranuloma venereum is resistant to the action of penicillin (Andrews). This virus as well as trachoma virus are acted upon by sulphonamides.

The handicaps of chemotherapy of virus infections are known and many claims made have not been substantiated. Sulphonamides are, however, effective against such viruses as those of lymphogranuloma venereum and trachoma and it is believed that penicillin has some virostatic activity against some of the larger viruses (Herrell; 1945). Against most of the small viruses penicillin has little or no activity.

(2) *Sensitivity of organisms*

In the following list the names of organisms sensitive and insensitive to penicillin are given. Most of the susceptible bacteria are gram-positive whereas gram-negative are resistant. Exceptions are gonococcus and meningococcus which though gram-negative are sensitive. Within the same species there are enormous variations. These differences should be borne in mind as the success of penicillin therapy depends on the sensitivity of the infective organisms and the concentration of penicillin at the site of infection.

The extraordinary minute quantities of penicillin required to inhibit the growth of bacteria may be judged from the fact that concentration of 0.13 unit per ccm (equivalent to about 1 in 13,000,000) is lethal to most strains of meningococcus; 500 units per mgm completely inhibit the growth of staphylococcus at dilutions of 1 in 30,000,000 and partial inhibition was produced with dilutions up to 1 in 160,000,000 (one grain in 2,000 gallons). In most of the clinical infections the effective blood level is 0.4 units per ccm, equivalent to 1 in 16,000,000.

(i) *Methods of determination.*—The methods depend on the measurement of zone of inhibition when the test organism is streaked on an agar plate containing a depot of penicillin. Growth is inhibited on an area surrounding the penicillin depot, the extent of this area showing the sensitivity of the organism. Fleming's gutter plate method consists of making a gutter by removing a strip of agar from the plate. The gutter is then filled with melted agar in which penicillin has been incorporated. The infecting organisms and the standard organism are streaked from the gutter to the edge of the plate, which is then incubated. The length along which the growth takes place is in inverse proportion to the sensitivity of the organism. Filter paper soaked in penicillin may be put in the gutter instead of melted agar with penicillin.

(ii) The following table gives the organisms which are susceptible and those which are insusceptible to penicillin in vivo and in vitro:—

Sensitive

Staphylococcus (albus, aureus)
Streptococcus (pyogenes, viridans, anærobic, non-hæmolytic)
Pneumococcus (*Diplococcus pneumoniae*)
Gonococcus (*Niss. gonnorrhoea*)
Meningococcus (*Niss. intracellularis*)
B. anthracis
Diphtheria group
Clostridia (tetani, novyi, perfringes, histolyticus, œdematis maligni, botulinum)
Streptobacillus moniliformis
Actinomyces
Spirochætes (relapsing fever, syphilis, yaws, vincent's angina, Weil's disease)
Spirillum minus (rat-bite fever)
Larger viruses (psittacosis, ornithosis) partly
Rickettsia partly
Besides these *B. subtilis* (hay bacillus), *Streptobacillus moniliformis, Erysipellothrix rhusiopathiae,* (erysipelodi; swine erysipelas), sarcina.

Insensitive

Enterococcus (str. fæcalis)
Non-pathogenic gram-negative cocci
Typhoid, paratyphoid group
Dysentery group; coli group
Vibrio choleræ
Proteus vulgaris
Pastuerrella (pestis, tularensis)
Pseudomonas group (Ps. æroginosa, B. fluorescense, ducreyi)
Hæmophilus group (influenzæ, pertussis)
Acid-fast group (tubercle, smegma)
Brucella melitensis
Friedlander's bacillus, (Klebsiella pneumoniæ)
Plasmodium vivax
Most of the viruses
Trypanosomes
Besides these yeasts, monilia, moulds, etc.

8. Penicillin in Therapeutics

Fleming (1946) has laid down a few simple rules for penicillin treatment:—

(1) Penicillin should only be used when the infecting organism is sensitive to it.

(2) Among the sensitive organisms there are some strains which are relatively less sensitive than others but in most cases the line of action is quite clear. Infection with sensitive organisms are controlled while those with insensitive ones generally are not. The sensitivity of the organism should be tested. Treatment, however, should not be delayed until bacteriological diagnosis is established any more than antitoxin should be withheld from a suspected case of diphtheria.

(3) Penicillin must come in contact with the organism by local treatment or through the circulation.

(4) The dose should be such that the concentration of penicillin attained should be sufficient to destroy bacteria and that this concentration should be maintained.

(5) The treatment should be persisted until the infection is controlled.

Concentration in blood.—On account of rapid excretion of penicillin its concentration in the blood and tissues rapidly falls. The following table shows concentration attained in serum during treatment with repeated intramuscular injections:—

SERUM TITRE IN UNITS PER CCM

Dose	½ hour	1 hour	1½ hours	2 hours
25,000	0.35	0.2	0.063	0.03
50,000	1.0	0.6	0.3	0.2
100,000	1.7	0.8	0.625	0.3

With continuous intravenous infusion 0.1 unit serum level per ccm is attained for each 100,000 units given per day. The same concentration is obtained with intramuscular drip

9. Mode of Administration

(1) *Local.*—It was originally observed that local applications of crude broth filtrates of cultures of *P. notatum* were beneficial in infected wounds. Purified preparations in form of sodium or calcium salts were then used for local treatment, and the calcium salt was found to be more satisfactory. Physiological saline or water solution containing 1,000 units of penicillin per ccm are suitable for local application and may be instilled through small rubber tubes into deep wounds. Higher concentrations should be used for infections with the less susceptible organisms. It is important to realize that solutions of penicillin should not be used merely for irrigation but should be instilled because in the former case the drug does not stay in contact with the infected wound long enough to permit bacteriostatic action. Injection into an abscess cavity after removing as much pus as possible is a good method of treatment. If pus is too viscid to pass through a needle it becomes liquified after one or two days of penicillin treatment. Infiltration of penicillin in strength of 1,000 units or more round an abscess sets up a barrier between the infected area and normal tissues and is useful. In such cases to prevent pain due to stretching, a local anæsthetic is combined with penicillin. Local sprays and wet dressings are also useful for surface lesions.

Prolonged contact essential.

Penicillin is locally applied to serous cavities and the meninges effectively. It has been shown that 25 mega units as injected into the blood produce the same effects as 500-1000 units given directly into the theca.

Penicillin can be ground with sulphonamides in a mortar in form of a powder each gram containing 5,000 Oxford units. A cream with lanette wax as base containing 1,000 units of penicillin per gram is also used for local application. Such a cream has been found to be very useful in the treatment of such minor conditions as superficial wounds, minor burns, sycosis barbae,

impetigo, etc. The cream may be applied directly, but it is better to use it spread thinly on a gauze. In the treatment of severe inflammatory lesions, however, satisfactory results are more likely to be obtained by a combination of local use with systemic therapy.

Local treatment with sprays or inhalations with masks has been successfully used in the treatment of lung conditions. By means of atomizers in which a jet of oxygen is led through the solution of penicillin to the patient who inhales it through a mask, the rate of flow can be controlled through a tap. The vapour is filtered free of larger particles and the patient inspires through the nose and expires through the mouth. In this way doses of 100,000 to 500,000 units dissolved in 3 ccm of water will be absorbed in half an hour. Lamellae are used for eyes and lozenges for infections of the mouth and throat. Dental cones are also used for insertion into the cavity after tooth extractions as a prophylactic measure, and for the treatment of dry sockets. Each cone should contain not less than 500 units and may also contain sulphathiazole or sulphanilamide.

(2) *Subcutaneous route.*—This route was not used because the impurity in penicillin solution not only produced considerable pain and discomfort to the patient but irregular absorption. Now that purer preparations are available this method is coming into vogue.

(3) *Intramuscular route.*—Penicillin can be administered by various methods.

(i) *Intermittent method.*—Twenty thousand units or more in one to two ccm of isotonic saline or redistilled water are given intramuscularly every two to three hours, a standard 22 gauge intramuscular needle, 2½ (6 cm) inches long being used. Local irritation sometime occurs but this is not serious; the only disadvantage is that eight injections are required in 24 hours. Besides this, the concentration of penicillin in the blood shows a sharp rise in the first hour following the injection and then falls low before the next injection is made. This is not desirable particularly in the treatment of severe infections.

The dose to be given is contained in 1 to 2 ccm of solution and this is taken up in a 2 ccm syringe. If necessary 0.5 ccm of 1/2 per cent anæsthetic is added to stop pain when it worries the patient. The buttocks and thighs are the best sites. In the buttock, the upper and outer quadrant of the gluteal muscle, along a line joining the top of natal cleft and top of the great trochanter is best. In the thigh inject over the great trochanter where there is a lymph space or into the vastus lateralis muscle which has no important structures underneath. Injection may also be given in the shoulder into the deltoid or triceps muscles.

(ii) *Continuous or intramuscular drip method.*—The object is to avoid repeated injections; 100,000 units or more dissolved in a litre may be used, but more concentrated solutions, *e.g.*, 100,000 to 500,000 *i.u.* in 100 ccm of physiological saline free from pyrogens are now advised. There is possibility of the needle being blocked and special care has to be taken to fix the needle in position in the muscle substance.

Various types of apparatus have been devised for this purpose, Broadbent type, Edwards type and Eudrip apparatus being examples. Some of these are mechanically operated either by electric or clockwork devices. A constant level of penicillin concentration in the blood is maintained by this method. The only disadvantage is that local irritation may be set up and an abscess may form.

Intramuscular drip is best given in the outer aspect of the thigh; this allows the patient considerable freedom of movement and the apparatus is available for inspection, to see that the tubing is protected from weight. A cradle is helpful and the tubing is led from its bottom.

Continuous administration has the disadvantage that it needs a somewhat complicated apparatus and the patient has to be permanently fixed to it. Hospitalisation is essential for this. Intermittent injections can be easily given and inspite of eight injections required in 24 hours, it is preferred. By increasing the dose of penicillin the interval between injections can be increased. The following table after Howard Hugh (1946) gives the dosage required with increasing time intervals:—

Time in hours	Preparations	
	Aqueous solution (units)	Oil & Wax suspension (units)
3	15,000	
4	30,000	
6	100,000	50,000*
8	150,000	100,000
10	200,000	120,000
12	300,000	150,000
18	500,000	250,000
24		300,000

The prolongation of effect of penicillin can be obtained by mixing it with such substances as beeswax, peanut oil, etc. or with the use of procaine penicillin.

(iii) *Beeswax-oil suspensions.*—Beeswax has been known to prolong the action of histamine, heparin, etc. It was found that if penicillin is combined in this material adequate concentrations of penicillin could be maintained in the blood of experimental animals for 6 to 12 hours and in man for 6 to 7 hours; penicillin was present in the urine for 20 to 30 hours. Mixtures containing 3 to 6 per cent of beeswax in peanut oil have been used for this purpose and assayable blood level was obtained for 7½ hours. In this way it is said a complete course of treatment can be given in an effective manner. Another advantage is that these mixtures even when kept at room temperature for as long as 62 days do not lose their potency. Calcium salt of penicillin is used for preparations of these mixtures and beeswax-sesame oil mixtures are even better. This combination should not be mixed with animal oils as a vehicle for penicillin as it is likely to form swellings after intramuscular injections.

Beeswax oil suspension is, however, not suitable when high concentrations of penicillin are quickly required in the blood for severe infections, septicaemia, etc. Besides, the results of absorption from this type of injection have been shown to be liable to considerable variations which make choice of dosage difficult.

(iv) *Procaine penicillins.* Procaine-penicillin G is a stable crystalline salt obtained by double decomposition of sodium penicillin and procaine hydrochloride and was first described by Sullivan et al (1948). The compound is only very sparingly soluble in water and has a potency of about 940 *i.u.* per mgm. It can be easily administered through an ordinary intramuscular needle and is painless. The compound is administered either as a suspension in water, a suspension in arachis oil, or as a suspension in oil with the addition of aluminium stearate. With suspensions in oil or water, a single dose of 300,000 units is capable of maintaining therapeutically effective levels for from 20 to 24 hours or longer. With a single dose of 300,000 units of the oily suspension with aluminum stearate, in a recent investigation, the effective blood levels were found to be maintained in 100 percent cases for 24 hours, in over 70 per cent cases for 48 hours and in a smaller percentage for even 3 days. It is thus evident that procaine penicillin

G in oily suspension with aluminium stearate is a highly suitable preparation for clinical use and gives slow absorption, economy in dosage, and prolonged therapeutic blood penicillin levels.

Local cold applications.—It has been shown that if cold is applied by means of an ice bag at the site of intramuscular injections, effective concentrations in the blood can be maintained for 5 hours as compared with 2½ hours without chilling, after a single dose. The frequency of penicillin injections can in this way be reduced to 2 or 3 instead of 8 or more in 24 hours.

(4) *Intravenous route*

Intermittent method.—Penicillin disappears from the blood stream more rapidly after a single intravenous injection than after a single intramuscular injection. Besides if this method is used a vein will have to be punctured 3 times each day. This method, therefore, is not convenient ordinarily for routine use. However, the initially high concentrations obtained with intravenous injections, makes the method extremely useful in the initial treatment of all such severe infections which carry a high mortality rate.

Continuous method.

Intravenous drip.—The dose of penicillin required for 24 hours is dissolved in a litre of isotonic saline solution or in 5 per cent glucose solution made in distilled water. The best veins to choose are the ankle veins. The ante-cubital veins are useful but as administration has to be continued for sometime the restriction to the patient's movements is considerable. If the needle has to be changed because of blocking or other causes, the site of injection should be changed.

Local irritation and thrombosis.

An 18 gauge transfusion needle is inserted into the vein (arm, ankle or back of the hand) and is anchored with adhesive plaster. A simple arm or leg splint or bandage is applied to keep the part in position. This method is fairly comfortable and avoids 8 separate punctures. Local venous irritation, however, at the site of injection may occur, particularly with batches of penicillin which contain impurities. If signs of irritation occur the site of injection should be changed at once. Heparin (3 units per ccm) has been combined with penicillin in intravenous infusions to prevent thrombosis or irritation but this is not considered advisable. If infusion is given at the rate of 35 drops per minute, the amount of heparin injected will not materially effect the clotting time of the blood.

Technique.—The technique of administration is as follows:—

To begin with 100 ccm of solution is allowed to run into the vein rapidly. after this the flow is so regulated that only 30 to 40 drops per minute are injected. In this way it will take one litre of solution from 8 to 10 hours to flow in and then the second litre is given; 100,000 to 500,000 *i. u.* can be given per day in 2 litres of physiological saline. This is the method of choice in the treatment of severe infections in which bacteraemia is present. By this route doses such as 300,000 *i. u.* to 1 mega units may be given without difficulty. Single doses are now not given by the intravenous route unless specifically indicated as with the purified penicillin now available, intramuscular injections of large amounts such as 0.5 mega unit cause practically no pain or discomfort. The rubber tubing used should be made of natural rubber as synthetic rubber tubing is said to inactivate penicillin.

(5) *Other routes*

Other routes.

(i) *Intrathoracic administration.*—In empyema, penicillin can be introduced directly into the pleural cavity and if necessary supplemented by systemic ad-

ministration. Fifty thousand units or more of penicillin may be administered daily by this route, the amount being dissolved in 40 to 50 ccm of physiologic saline solution.

(ii) *Intra-articular administration.*—In suppurative disease of joints 20,000 units or more dissolved in 10 ccm of isotonic saline solution may be instilled into the joint after aspiration. As penicillin remains in the joints for longer periods, according to some for 24 hours, it is not necessary to repeat injections oftener.

(iii) *Intrathecal administration.*—Penicillin does not diffuse into the cerbrospinal fluid after systemic administration. Even 100,000 units by the intramuscular or continuous intravenous infusion do not produce an assayable level of penicillin in the cerebrospinal fluid of patients who have no meningitis but significant amounts are present when meningitis is present. In purulent meningitis, however, effective concentrations can be obtained. In neurosyphilis, after one or two such large doses as 300,000 to 500,000 units, concentrations of about 0.02 unit per ccm were obtained in the cerebro-spinal fluid.

Ten to twenty thousand units of penicillin can be directly introduced into the spinal canal, the whole being dissolved in 10 to 20 ccm of isotonic saline solution. Either the sodium or calcium salt may be used. Five thousand to 10,000 units may be instilled once or twice daily into the ventricles of the brain if a small tube has been left in place after operation. It is, however, liable to set up irritation (see toxic effects). Sterility of the solution to be injected is of the utmost importance.

(iv) *Infusion by bone marrow.*—Penicillin can be introduced into the sternum or clavicle or tibia or femur and as much as one litre of solution can be given by this route in 16 hours. The technique of injection is the same as for continuous intramuscular drip and the same apparatus may be used. This route is used primarily in the treatment where suitable sites are not available for intramuscular injection, as in extensive burns and mutilations, shock etc. and in very small infants.

(v) *Rectal administration.*—This route would appear to be unsound because of penicillinase-producing bacterial flora. Appreciable blood levels of 0.8 units per ccm were, however, obtained by some workers by a simple oil of theobroma suppository containing 300,000 to 1,000,000 *I.U.* though the results were irregular; others have obtained negative results.

(vi) *Oral route.*—Owing to rapid inactivation in the acid of gastric juice a large proportion of the penicillin is destroyed when given by mouth. Oral therapy is effective in achlorhydria. It has been claimed that penicillin given in simple saline solution produces high concentrations in the blood. Methods have been adopted by either protecting the penicillin or keeping the gastric contents as near pH 6 as possible. Little success was obtained with salol coated pills or capsules but suspensions in oil were more successful. Enteric coating has been tried.

Simultaneous administration of a buffer has given satisfactory results and so also a mixture with raw egg. Reports with use of sodium or potassium citrate have been favourable but too much alkali also destroys penicillin. By using aluminium hydroxide gel, magnesium trisilicate or magnesium hydroxide, a neutralising action combined with absorption and slow release of penicillin is obtained. Tablets of 25,000 or 50,000 units of calcium penicillin buffered

with 0.5 gm sodium citrate have been claimed to give satisfactory results and are available in the market. Administration of the sodium or calcium salts 100,000 units together with 30 ccm of aluminium hydroxide has been found to give satisfactory results. Kolmer (1948) believes that the most satisfactory method of oral administration consists in the administration of tablets of 50,000 units of the calcium or potassium salt, the latter being preferred, along with 0.5 to 1 gm of sodium benzoate for the reduction of renal excretion, and without the addition of a buffering agent.

(vii) *Penicillin spray (Aerosol Therapy).*—Systemic therapy by inhalation of a spray is possible and after inhalation of 25,000 units in 25 ccm of physiological saline, a concentration of 0.62 units per ccm of blood was obtained for ½ to 3 hours. Penicillin can also be given in form of inhalation in such diseases as bronchitis or bronchiectasis, the patient inhaling it throughout the day. One ccm of physiologic saline solution containing 25,000 units of penicillin can be nebulized or inhaled by a patient in 10 minutes. This can be done three times a day and as much as 75,000 to 80,000 units can be administered by this method. Penicillin in form of a snuff (5 parts of penicillin by weight, 5 parts of menthol and 9 parts of lycopodium) have been used. Except in very superficial type of nose infections this method does not give satisfactory results.

(viii) *Intra-arterial route.*—This route of administration of penicillin has recently been shown to be safe, economical and effective in treating severe infections of the extremities. A 20 ccm syringe containing 10 ccm of aqueous solution 50,000 units, and a No. 20 needle is employed. For the lower limb the site of election is the femoral artery at the inguinal crease, and for the upper limb, the brachial artery at the elbow.

10. Effectiveness Against Infections

Introductory.—In the struggle between the tissues of the body and the infecting organism, two things might happen. Either there is a complete recovery, or a low grade infection may persist which may produce periodic attacks of ill health later in life. If thorough chemotherapeutic treatment is given, the infection is often completely eradicated, but if it is not, there is tendency for the infected individual and the organism to become symbiotic; the infection in that case may occasionally lighten up and produce symptoms but otherwise remains dormant. The chemotherapeutic effects are mainly dependent upon the size of the dose and the duration of treatment; recurrences have been shown to occur in inverse ratio of these two factors. In the application of chemotherapy, therefore, both the size of the dose and the duration of treatment are important considerations.

Another important point to be considered is how much damage has been done to the tissues before specific treatment is applied. If much tissue damage has occured, the result so far as the patient is concerned is not satisfactory. It is essential, therefore, that chemotherapy should be started early so that serious damage has not been done to the tissues and resulting complications have not set in.

The action of penicillin and sulphonamides is specific against certain infective organisms by virtue of their bactericidal and bacteriostatic action and their power of penetration into the tissues. It is important to determine how far the infection has proceeded and how the patient is responding to it. The object of application of chemotherapy should be to assist the patient in overcoming the infection.

Penicillin therapy.—The advantages which penicillin possesses are that it has a powerful action against a group of serious bacterial infections, it is non-toxic, it is active in presence of pus and body fluids, and it can be administered in adequate doses to maintain an effective anti-bacterial level in the blood and tissues. Before giving penicillin it should be determined whether the infecting organism is sensitive to penicillin or not, but this is not necessary if delay is dangerous to the patient. The physician may often have to give penicillin after clinical diagnosis only. In such cases the clinical course of the disease should be very carefully watched. If response is satisfactory it may be felt that a penicillin sensitive organism is being dealt with. If, however, after the correct dosage, the clinical response is not satisfactory, the diagnosis is probably incorrect. Within three days of the start of penicillin therapy or even earlier, it should be possible to determine whether the treatment should be continued or not.

If the patient is showing satisfactory response to therapy, it has to be determined how long the treatment should be continued. In this, guidance has to be obtained from the improvement in the condition of the patient and negative bacterial cultures. It may be reiterated here that in chemotherapeutic treatment of infections, early diagnosis and early treatment are of utmost importance.

Dosage for systemic therapy:—The doses given when supplies were limited were 15,000 units every three hours, or 120,000 units in 24 hours by continuous drip method intravenously or intramuscularly. This minimum dosage, however, allowed little margin for individual variations in patients and the resistance of various strains of bacteria. The effective blood level is 0.1 unit per ccm, and with 15,000 units every three hours, only a concentration of 0.065 units is reached and with 20,000 units 0.09 units per ccm. Consistent results are only achieved with higher dosage and less than 20,000 units (equalling 12.0 mgm or 1/5 gr.) of penicillin should not be used. The tendency now is to use larger doses and even 50,000 units or more every three hours is not considered too much to control an active infection. Routine dosage and its duration should be carefully worked out for different infections. The treatment should not be stopped too soon as this leads to relapses. It should, be continued after the patient appears to have overcome the infection. In general practice, 100,000 to 300,000 units given twice daily, produce and maintain the required concentration in blood and tissues. Doses as large as half to one million units can be easily given intramuscularly without discomfort. Penicillin should be started before bacteria have become established as its effect in primary stages of an infection is much stronger than when the infective process has taken root. If early diagnosis is established, a dose of 200,000 units or more up to 500,000 units, followed by a second dose of 100,000 to 300,000 units a few hours later, have remarkable effects in overcoming the infection.

Acute and Chronic infections:—If penicillin is adequately exhibited early, clinical evidence of the arrest of an acute infection soon becomes obvious. In such cases 100,000 to 300,000 units every 3 to 6 hours produces remarkable effects. It is not necessary to wait for bacteriological reports before starting treatment. Chronic infections, however, are more resistant and need more intensive and prolonged treatment. Surgical measures, if indicated, should be implemented without delay as penicillin does not supplant surgery. If applied properly and liberally, penicillin will prevent abscess and slough formation, but if an abscess has formed it should be opened and drained. The use of penicillin before, and sometime after application of surgery, is of great prophylactic value. Surgical measures can be undertaken with great advantage in such conditions as osteomyelitis when penicillin is being exhibited.

The effect of penicillin often is dramatic and is evident in a few hours as shown by fall of temperature, disappearance of pain, etc. In other cases high temperature may persist and only come down at the end of penicillin treatment. Fever as such, therefore, does not indicate failure of penicillin treatment.

(1) Infections of blood stream and heart.

Bacteraemia and Septicaemia.

(i) *Septicaemia and pyaemia*:—The presence of pathogenic bacteria in the blood stream is an indication for immediate intensive and prolonged exhibition of penicillin. A proper bacteriological investigation is essential and can give useful indication of progress of treatment, but administration of penicillin should be started without waiting for the result. The degree of suceptibility of the organism will give a clue to the dosage. Bacteraemia or septicaemia were difficult to treat till the discovery of sulphonamide drugs, and even with these compounds, micro-organisms become resistant. Further, these drugs are toxic. Staphylococcal bacteraemia is not amenable to treatment with sulphonamides but is effectively controlled with penicillin. Streptococcal bacteraemia in which sulphonamides have failed has been brought under control, as also bacteraemia due to *Diplococcus pneumoniae, Neiss. intracellularis,* anaerobic streptococci, micrococci, micro-organisms of the genus proteus and salmonella.

Bacteraemia not associated with subacute bacterial endocarditis rapidly yields to penicillin treatment. There is no contraindication to the use of penicillin in the treatment of bacteraemia and even very severe cases may recover. It is essential, however, that the concentration of penicillin in the blood be maintained at a constant and effective level during the first few days of treatment. Continuous intravenous drip is the method of choice and large doses should be given to prevent resistance developing in bacteria. Virulent bacteria may remain in the blood stream for sometime inspite of apparent clinical improvement. This has been demonstrated by frequent bacteriological examinations. Treatment should be continued for 4 or 5 days after bacteria have disappeared from the blood and temperature is normal, though injections may be less frequent (300,000 units morning and evening).

(ii) *Subacute bacterial endocarditis*:—The great majority of organisms responsible for this hitherto fatal disease, are sensitive to penicillin though in varying degrees, and with the adequate use of penicillin the infection may be eradicated in as many as 80 to 90 percent of the cases. A certain percentage of these, however, succumb as a result of extensive damage already caused to such vital organs as the heart, kidneys etc. The earlier the disease is treated, the higher the chance of cure. In old standing cases and in those who show signs of extensive damage to vital organs, the ultimate outlook is not so encouraging.

Dosage and duration of treatment.

There is no uniformity of opinion with regard to dosage of penicillin and duration of treatment. The procedure now generally approved is to give 100,000 units of an aqueous solution intramuscularly every 2 hours for at least 8 weeks after all signs of active infection have subsided. Such a dosage schedule continued over such a prolonged period has been found in practice to yield the best results and the smallest relapse rate.

Delay in treatment diminishes chances of cure and inadequate doses are naturally prejudicial to success and may give rise to serious complications, besides development of resistance in the infecting bacteria. Early diagnosis of the disease is, therefore, of utmost importance. The blood culture usually be-

comes negative within a few days after treatment is started, but the fever may persist longer and usually comes down to normal within a week or so. If dosage is inadequate, fever returns and relapse occurs.

Prophylaxis.—In patients suffering from intrinsic cardiac lesions, penicillin should be employed as a prophylatic measure before and after surgical procedures such as extraction, of teeth, etc. which are followed by transient bacteraemia and subsequent subacute endocarditis. Administration of 500,000 units daily for not less than 4 days have been suggested.

Acute bacterial endocarditis. This is a complication of septicaemia. The line of treatment is the same as in case of subacute endocarditis, *i.e.*, at least 10 to 20 mega units daily for not less than 8 weeks. Chances of recovery have been greatly improved by such intensive exhibition of penicillin.

When sulphonamides are combined with penicillin, it is desirable to maintain the concentration of the former drug in the blood at from 14 to 18 mgm per 100 ccm. Sulphonamides can be given orally or they can be incorporated in the penicillin solution.

Suppurative pericarditis reacts to treatment with penicillin. After the pus is aspirated from the pericardium, 100,000 units or more of penicillin in 5 to 10 ccm of physiologic saline are injected into the pericardial sac.

Infective Phlebitis:—If phlebitis is secondary to infection, dramatic results are obtained by penicillin therapy. The danger of cavernous or lateral sinus thrombosis from a carbuncle of the face, mastoiditis or a dental abscess is reduced. If this complication arises, 100,000 units should be given every three hours intramuscularly, or 0.5 to 1.0 mega unit may be given during 24 hours by intramuscular or intravenous drip.

(2) Infections of the central nervous system and special organs.

Sepsis inside the cranial cavity is a serious condition because it raises pressure inside this rigid cavity and because the cerbro-spinal fluid has a poor defence against infection. Discovery of penicillin and sulphonamides has revolutionized the treatment of sepsis within the skull. Many infective processes start from infections in the middle ear and sinuses, and on account of improvement in the treatment of these conditions, intracranial infection is less frequent. Osteomyelitis of the skull (staphylococcal) may occur from infection of sinuses, especially of the frontal sinus. Formerly it was practically always fatal. Systemic use of penicillin has produced a remarkable decrease in the mortality rate in this condition. In the treatment of cranial infections, therefore, the primary condition should not be neglected.

Penicillin unfortunately does not pass the biood brain barrier and it has to be given directly into the cerebro-spinal fluid. A single dose can, however, maintain effective concentration for 24 hours. Penicillin if not pure, produces irritation, but pure penicillin (1,000 units or more per mgm) produces no ill effects. Injections are usually given by lumbar puncture but if the canal is blocked the intracisternal route may be used.

Accidental contamination of penicillin with a nonsusceptible organism such as *Ps. pyocyanae* may produce death. Care must therefore be taken that the syringe, skin, etc., are adequately sterilised.

(i) *Meningitis.* Penicillin is effective against a number of micro-organisms which produce meningitis. Meningococcal meningitis produced by *Neiss. intracellularis* reacts to treatment with sulphonamides (sulphapyridine, sulphadiazine

and sulphamerazine) and although there has been a considerable reduction, the mortality is still high. It should be borne in mind that sulphonamides pass into the cerbro-spinal fluid from the blood but penicillin does not, though there is some evidence that in inflamatory conditions it does go through. Penicillin 10,000 units in 10 ccm saline should also be injected into the cerebro-spinal fluid by lumbar, cisternal or ventricular punctures. All collections of pus anywhere in the skull should be drained off. Fibrinous pus occurs on the surface of the cortex, and for this penicillin is applied locally by means of a flexible catheter passed over the surface of the brain and as it is slowly withdrawn, penicillin 500 units per ccm is distributed over the surface of the cortex.

Pneumococcal type.

In pneumococcal meningitis good results have been obtained with penicillin. Combination of penicillin with sulphonamides gives better results. For pneumococcal meningitis, the routine of treatment is the same as with meningococcal meningitis; penicillin, however, is combined with sulphadiazine or sulphamerazine which may be given either orally or intravenously to get concentration in the blood up to 14 to 18 mgm per 100 ccm. The sulphonamides should be continued as long penicillin is given, unless toxic reactions occur. The course of treatment in this form is longer than in case of meningococcal meningitis.

Staphylococcal type.

Staphylococcal meningitis is not common and was occasionally cured with sulphonamides. Penicillin alone is quite effective in its treatment but it is better to employ sulphadiazine along with penicillin, for the synergistic effects.

Streptococcal Type.

In streptococcal meningitis, penicillin is useful particularly when the hæmolytic streptococcus is present. Here again it is advisable to use sulphadiazine in combination with penicillin. Cases of recovery in *Str. viridans* infections have also been recorded, as also in infection with anærobic streptococcus when sulphonamides and other agents have proved useless. Some cases of infection with *H. influenzae* have also benefitted.

In the treatment of meningitis penicillin should be given early to maintain effective concentration in all parts of the cerebro-spinal pathways in the cerebrospinal fluid for a sufficient length of time. Penicillin should, therefore be given intrathecally as well as systemically. The treatment should be immediately started and should be carried out for seven days or longer if the patient does not recover.

(ii) *Wounds of brain and brain abscess*

Penicillin has also been used in cases of brain abscess following trauma to the head and has sometimes been combined with sulphadiazine after surgical drainage has been established. The organisms present are usually *Staph. aureus* and *str. pyogenes.* In recent wounds of the brain (up to 72 hours old), calcium penicillin in form of a powder (5,000 units of penicillin combined with one gram of one of the sulphonamides) applied locally is useful. In old wounds of the brain, after excision and closure, sodium or calcium penicillin solution (500 to 1,000 units per ccm) are instilled through tubes left in at the operation. Pure penicillin given in this way is not irritant. Sulphathiazole is not recommended locally in form of a powder as it may cause irritation on direct application.

(iii) *Infections of the eye, ear, nose and throat*

Conjunctivitis.—Penicillin has been used in the treatment of conjunctivitis produced by various organisms, *e.g., Staph, aureus, Str. pyogenes, Diplococcus pneumoniae* and *Neiss. gnorrhoeae* in form of an ointment (30,000 units per gm) and drops (2,500 units per ccm). Drops are put in every hour or even few

minutes as they are easily washed away by the lachrymal secretion. Lamellae cause discomfort. Pure penicillin is better tolerated than the ordinary form. The belief held that penicillin does not diffuse readily into the tissues of the eye is being modified, as satisfactory results have been obtained in extensive orbital cellulitis by giving penicillin systemically. Subconjunctival injections in doses of 25,000 or 50,000 units in 0.5 ccm of saline give effective concentration in the aqueous and the vitreous humors. In purulent conjunctivitis drops put in every few minutes for the first quarter hour and then every half hour for 2 to 3 hours give relief.

Keratitis & other conditions.—For superficial ulceration, conjunctivitis, keratitis, acute or chronic blepharitis (*Staph. aureus*), and dacryocystitis due to sensitive organisms, a cream (5,000 units per gram), or a physiological saline solution (1,000 to 5,000 units per ccm) are recommended for local application every one to two hours and continued till there is definite clinical response. Crusts in blepharitis may be removed with hydrogen peroxide. In severe infections local treatment should be combined with systemic administration of 100,000 units per day. Hypopyon ulcer is treated by local application of ointment.

Penicillin is of little or no value in the treatment of infections of the central nervous system and the eye produced by *Myco. tuberculosis.*

In external otitis, penicillin is useful as a local application in form of cream or in aqueous drops for a few days. Oily drops (1000 to 5000 units per ccm in castor oil) have been used. Even infections with *B. proteus,* which is insensitive to penicillin, have reacted.

In acute otitis media in young children, two three injections of 50,000 to 100,000 units at intervals of 4 to 6 hours are usually sufficient but treatment is continued for 2 to 3 days. For older children and adults 200,000 to 500,000 units are given, often with immediate relief. If the drum is perforated penicillin powder is blown into the external ear. If a solution is used 1 ccm of a solution containing 2000 units or more is instilled into the canal keeping the head tilted and sterile gauze is then plugged in afterwards. For less severe cases, tablets of buffered penicillin 50,000 to 100,000 units may be given orally every 3 hours day and night.

In chronic otitis media mixed infection is often present and penicillin may be combined with sulphonamides. Drops containing both substances are instilled into the ear.

In mastoiditis in acute stages, penicillin therapy started early may prevent surgical intervention. Penicillin is given in doses of 100,000 units intramuscularly every 3 or 4 hours and four doses give considerable relief. The patient should, however, be carefully watched for masking of symptoms of intracranial complications.

In chronic cases, penicillin therapy may make cortical mastoidectomy possible instead of the radical operation, thus saving the hearing of the affected ear.

Rhinitis.—The common cold is usually a virus-infection and penicillin has no effect in this condition, but the virus is generally accompanied by secondary invaders and these are acted upon by penicillin. This accounts for relief obtained or even abortion of a cold by sprays. Congestion is decreased by a previous aqueous spray of ephedrine and this also increases penetration of penicillin and decreases nasal discharge. Penicillin spray contains 10,000 units per ccm and if desired this can be combined with ephedrine hydrochloride.

Sinusitis. In acute suppurative sinustis, local as well as systemic administration of 200,000 to 500,000 units is necessary. A single dose may bring relief but it should be repeated after 8 to 12 hours. In later stages pus should be evacuated and lavage and instillation of penicillin instituted. According to some, penicillin has deleterious effect on the cilia of the nasal mucous membrane and they prefer weaker solutions containing 250 units of penicillin per ccm. Penicillin therapy is of prophylactic value against spread of infection during surgical interference in nose, *e.g.,* removal of polypoid, growths, etc.

Throat.—Pharyngitis and laryngitis respond well to local application if the infecting organism is sensitive. In the early stages vigorous local use may abort an attack. Sprays are better than use of lozenges. In quinsy, massive doses of 200,000 to 500,000 units twice daily prevent abscess formation. Diphtheria bacillus is sensitive and 100,000 to 300,000 twice daily are effective. The diphtheria toxin, however, is not neutralized and anti-toxin has to be administered. Penicillin has also been used in the treatment of Diphtheria carriers, sometime in combination with sulphathiazole.

In septic sore throat and acute tonsillitis produced by *Str. pyogenes,* sulphonamides are as a rule successful. If these are not well tolerated or are ineffective, penicillin gives satisfactory results. The preparation used consists of one part by weight of penicillin, five parts of menthol and 94 parts of lycopodium; the powder is applied six times per day.

(3) Infections of respiratory tract

In acute infections of the chest 1,000,000 to 2,000,000 units are usually sufficient, spread over a period of 13-14 days. Besides this, local injections of penicillin into the pleura in doses of 20,000 to 40,000 units repeated every 24 hours may be given if necessary. Penicillin is absorbed slowly from the pleura into the blood, remaining there for 24 hours. If 120,000 to 240,000 units are injected into the pleura, sufficient concentration may be maintained in the blood for 48 hours.

Pneumonia.—For treatment of pneumonia due to *Diplococcus pneumonia,* sulphonamides give satisfactory results. They have not been effective in pneumonia produced by staphylococci and streptococci. Penicillin should be administered by the intramuscular route or intravenous drip method, the former being generally preferred, in doses of 200,000 to 500,000 units twice or thrice daily; recovery occurs in 2 or 3 days. In less acute cases, penicillin may be administered in tablet form, 100,000 units every three hours, day and night. Uncomplicated cases do not require more than 3 or 4 days treatment, but longer treatment is necessary in case of pneumonia due to staphylococci, streptococci and susceptible viruses. Intramuscular injections are preferred in those cases in which there is marked congestion or if complications are present. If bacteraemia due to susceptible organisms is present it is advisable to give penicillin by the intravenous drip method for the first two days at least. In broncho-pneumonia a mixed infection is often present and a combination with sulphonamides is considered desirable.

Briefly though pneumococcal pneumonia responds satisfactorily to sulphonamides penicillin is the drug of choice. It is also indicated (a) when there is no response to sulphonamides within 24 hours or when there is sensitivity to sulphonamides and toxic symptoms are apparent or there is resistance to these drugs; (b) when such complications as leucopoenia, liver, kidney or heart disease are present.

Bronchitis & Bronchiectasis.—In bronchitis and bronchiectasis a standard glass nebulizer operated by compressed air is useful. Patients can inhale one ccm of nebulized saline containing 2,500 to 5,000 units of penicillin in 10 minutes. This should be repeated three times an hour throughout the day and in this way 75,000 to 80,000 units can be inhaled.

Empyema and Lung abscess.

Penicillin is more useful in the treatment of acute bronchitis than in the chronic form, where probably fibrotic changes have occurred.

In bronchiectasis, where the patient has fever and the organism is sensitive, penicillin is very useful, and should be given simultaneously by inhalation and by intramuscular injections. Daily injections of 300,000 units are adequate to supplement aerosal therapy. Inhalations of 25,000 to 50,000 units are given three or four times during the day and once or twice during the night. It may be beneficial to give streptomycin along with penicillin.

Penicillin is useful in the treatment of pneumococcal, staphylococcal and streptococcal empyema, and lung abscess. In the former, intra-thoracic instillation of penicillin is combined with systemic administration, but often intrapleural administration alone may be successful. For intrathoracic administration following thoracentesis, 40,000 to 50,000 units dissolved in 40 to 50 ccm of physiologic saline are injected and left in the pleural cavity, the procedure being repeated once every 24 to 48 hours. When surgical drainage has been previously established, penicillin is introduced through the drainage tube which is clamped for several hours in order to prevent the penicillin from escaping from the pleural cavity.

Treatment of empyema is satisfactory if early diagnosis has been made. If, however, pus has been present for days or weeks, penicillin injections should first be given to overcome the infection and then surgical drainage is established if necessary by resection of a rib.

In lung abscess the sensitivity of the organism should first be tested. Generally, in staphylococcal abscesses, penicillin is useful, and in streptococcal infections results are variable; some are cured others are not. If bronchiectasis is also present, results are not good and if multiple infection is present results are variable.

Lung abscess.—Penicillin 40,000 to 50,000 units is given intramuscularly every three hours. If the abscess is drained surgically, solutions containing 50,000 units of penicillin are instilled directly into the drainage tube two or three times a day. Even in unopened abscess, penicillin may be introduced into the cavity, the needle being guided by X-ray.

In preparation of patients with suppurative disease of the lung or tumour for surgical operation, penicillin is generally given 4 days to 5 days before the operation as prophylactic.

(4) Infections of alimentary tract

Pyorrhaea.

Mouth and tongue.—In pyorrhoea alveolaris, pockets of pus are formed by deepening of the parodontal sulcus. These are not healed by penicillin as they become reinfected after the treatment is stopped. In dental surgery penicillin is useful in preventing spread of infection locally and into the blood. For this purpose 50,000 to 100,000 units are given quarter of an hour before extraction of septic teeth or other operations. A dental cone made from a paste containing penicillin and sulphonamide is inserted into the socket after penicillin in form of lozenges.

Glossitis.—Septic ulcers of the mouth and tongue respond to penicillin treatment and intensive systemic therapy is effective for acute parenchymatous glossitis; vincents angina has been successfully controlled by local use of penicillin in form of lozenges.

Acute abdominal conditions.—As *E. coli* is often concerned in infections of the gastro-intestinal tract penicillin is generally of little value. Penicillin sensitive organisms are, however, responsible for some infections and the elimination of secondary infecting streptococci of high toxicity by penicillin therapy is beneficial. In such conditions as ulcerative colitis, however, where non-susceptible organisms are concerned, penicillin therapy is doomed to failure. In acute cholecystitis, streptococcal infection is common, and in early stages intensive penicillin therapy must be employed as curative and later it may supplement operative treatment with advantage. In acute appendicitis, streptococcal infections may be present or predominant and here penicillin therapy is of value. It is particularly useful as adjuvant to operative treatment. In peritonitis due to streptococci, staphylococci and gonococci, massive dosage gives satisfactory results. Many strains of B. *typhosum* are sensitive to penicillin in vitro but clinical typhoid is not influenced by penicillin though some workers have obtained good results by its combination with sulphathiazole.

(5) Bone and joint infections

The crippling effects of bone disease have been almost entirely prevented by penicillin therapy, provided early diagnosis is made and treatment is immediately started. This prevents abscess formation, bone destruction sequestration and metastatic abscesses. Penicillin is useful in the treatment of acute osteomyelitis if given before thrombosis of the vessels has occurred. Better results are obtained if sequestra are removed by operation and penicillin is given at the same time. Spreading osteomyelitis of bones of the skull and face reacts to penicillin treatment. If surgically treated it is desirable to combine local therapy with systemic use of penicillin. The tendency is to use massive doses in acute haematogenous infections at the outset, a total dose of 400,000 on the first day, 200,000 on the second and 100,000 on subsequent days being favoured. At the same time general care of the patient, immobilisation, prevention of deformity and evacuation of subperiosteal or soft tissue abscess is very important.

Chronic osteomyelitis. Penicillin is useful in preventing attacks of chronic recurring osteomyelitis which may follow the acute disease. If early and adequate amounts of penicillin are given in acute osteomylitis, it is possible to eradicate this disease before foci become established. Once these are established, they are a constant source of trouble. In chronic osteomyelitis both systemic and local treatment is desirable along with saucerisation of the focus, immobilisation and primary closure of the wound.

Acute pyogenic-arthritis.—In acute suppurative lesions of joints (due to streptococci, staphylococci and pneumococci) it is desirable to aspirate 5 to 10 ccm of the fluid and instill 50,000 to 100,000 units of penicillin in an equal volume of physiological saline. Penicillin should not be repeated till after 48 hours; 80,000 to 100,000 units are given by the intramuscular route or intravenous drip. It is important that penicillin be given early before irrevocable damage has been done to articular surfaces and structures and this may take place in the course of a few hours. If the treatment is started early, 2 or 3 injections suffice but it should be continued till conditions become normal. If

damage has occured to the cartilage, ankylosis will occur. In tubercular joints where secondary infection has occured, penicillin is useful in controlling the latter.

In hæmarthrosis, aspiration should be followed by direct injection of penicillin into the joint under very rigid aseptic precautions to prevent infection.

Prophylactic use of penicillin in connexion with surgical operation of bone has given very satisfactory results, especially in orthopoedic surgery when the patient has a septic focus. *Prophylactic use.*

Compound fractures. For prophylactic purposes in compound fractures, it is advisable to give 20,000 units of penicillin every three hours intramuscularly for at least five days, in addition to the local application of sulphonamide compound powders. If infection is already established, give 20,000 to 40,000 units every three hours.

(6) Infections of the skin

Penicillin is useful where infective organisms are sensitive to it, *e.g., Staph, aureus, Str. pyogenes,* etc. Such common infections are impetigo, boils, carbuncles, erysipelas, pustular folliculitis, sycosis, blepharitis, etc. Less common are anthrax and erysipeloids (the bacillus of swine erysipelas). *Ery. rhusiopathiae, T. pallidum* and *Bor. vincents* all are *sensitive.* Fungi and coliform organism which infect skin are insensitive. Local application of penicillin is useful in the majority of cases where infection is superficial but in those cases where it is deep seated as in anthrax, boils, carbuncles, erysipeloids, etc., penicillin cannot reach the organism, it is not effective and it may produce local irritation.

Locally, solutions of sodium or calcium salts (500 to 1,000 units per ccm) are applied by means of sprays 2 for 3 times a day. This method is more economical than application of creams, but needs a somewhat complicated apparatus. A cream containing 27 per cent of arachis oil in water with 13 per cent Lanette wax SX as emulsifier is an excellent preparation. If patient is prescribed the cream, he is instructed to keep it in a cool place and apply it with a knife which is sterilized by dipping in boiling water before it is put in the cream pot. The cream is best applied twice daily, *i.e.,* morning and evening. It should be remembered that penicillin cream contains water and retains activity for a shorter period than the ointment. If there is no response within a week, it is not likely to do good.

Penicillin is effective in cases of eczema with secondary infections.

Boils & carbuncles.—In boils and carbuncles, penicillin is quite effective. Sometimes, solutions containing penicillin are injected into the deeper layers of tissues around boils and carbuncles. This method, however, is seldom necessary as most of the cases respond to systemic use of penicillin in usual doses of 100,000 to 300,000 units twice daily for 2 or 3 days. A single dose of 500,000 units may be given to arrest the development of boils. Two intramuscular injections of 500,000 units daily, if given early, will arrest development of a carbuncle.

For injection into the tissues, a saline solution containing 250 to 500 units of penicillin is the best. Ointments containing 400 to 500 units of penicillin per gram have been found to be satisfactory for local application.

Impetigo and Sycosis Barbae.—After removing the crusts penicillin ointment is applied locally in impetigo once or twice a day; marked improvement is noticeable in 8 or 9 days. Penicillin is also used systemically for these infections. In sycosis barbae, the infecting organisms are *Staph. aureus* and *Str. pyogenes;* crusts are removed before local application of penicillin; infection may not be eradicated in all cases.

Cellulitis.—In severe cases of spreading cellulitis with bacteraemia, good results are obtained with penicillin and erysipelas is also benefitted. The skin is infiltrated with 2 per cent procaine solution, pus is evacuated by aspiration and penicillin is injected through the same needle, 2,000 to 20,000 units being given locally according to the size of the lesion. Aspiration and injection of penicillin are repeated every two days. It is possible that intensive systemic therapy with penicillin may do away with surgical interference. Cellulitis of the fingers and neck is benefitted. Recurrent cellulitis of the face, though of streptoccocal origin, did not improve much after full courses of penicillin for 5 to 7 days. Sulphonamides have proved useful in pelvic cellulitis (acute and sub-acute) but not in the type due to anaerobic organisms. For the acute form, a full 7 days course of penicillin is recommended, 40,000 units or more every 3 hours. If suppuration occurs, local injection of 5,000 units of penicillin is indicated.

Ludwig's angina.—Penicillin is effective in the treatment and prevention of severe cellulitis of the neck (Ludwig's angina) and other parts of the body where the infecting organism is usually the hæmolytic streptococcus. Penicillin in large doses (1,000,000 units in 72 hours) may be life-saving. Ludwig's angina is generally due to dental infection in a large number of cases. The infection is usually multiple, the organisms isolated from these cases being streptococci, staphylococci, vincent's spirilla, micro-aerophilic streptococci and gas producing bacteria. All these are controlled by penicillin without surgical operation. If a large abscess is present it should of course be opened and penicillin should be given systemically.

Lymphangitis and Lymphadentis—if due to a septic focus, respond well. Abscess formation in lymph glands is prevented by early treatment with penicillin.

Parotitis and Scarlet fever.

Postoperative parotitis is improved with penicillin but not epidemic parotitis. In the treatment of scarlet fever, penicillin has been tried with unsatisfactory results.

Infected wounds and ulcers.—Local application of penicillin to wounds infected with such gram positive pathogens as clostridia, staphylococci. streptococci and cornybacteria is useful. If gram negative organisms are also present they are not affected and may even increase in number; continuation of local and systemic treatment is best in such cases. Penicillin is useful in post-operative infection of wounds after all kinds of surgical operations. It is also useful in phagedenic ulcers and tropical sloughing phagedena in which fusiform bacilli are present. The ulcers clear up with local dressing with penicillin; both fusiform bacilli as well as certain spiral organisms are sensitive to penicillin. Severe infections of the hand react well to penicillin.

Burns.—Penicillin has been largely used in the treatment of burns of all degrees with good results. Febrile patients were given 20,000 units intramuscularly every four hours. A generous application of penicillin in Lanette wax SX is effective if applied for 24 hours and in some cases it may be applied for 48 hours, the part being then covered with gauze to prevent loss of body fluid especially if the surface is large. Infecting organisms, such as streptococci and staphylo-

cocci, disappear rapidly. Penicillin is also very useful during grafting operations after burns. Penicillin therapy may produce sterility of the surface but sometime difficulties arise owing to wound becoming infected with penicillin insensitive organisms. It is best to apply penicillin only till the active bacterial invasion is controlled.

Cutaneous syphilis and small-pox.—Penicillin has been used successfully in the treatment of cutaneous syphilis.

Penicillin is useless in small-pox but it may be useful in secondary infections complicating the viruses of small-pox and chicken-pox.

(7) Genito-urinary infections

E. coli plays an important part in infections of the urinary tract and utility of penicillin would appear to be limited. It is, however, of considerable value in infections of the kidney and bladder and their ducts. In acute streptococcal nephritis in children 50,000 to 100,000 units 2 or 3 times a day are useful. In cystitis, secondary staphylococcal infections associated with catheters and suprapubic cystostomy, are controlled. In cases with sudden acute onset, a few massive doses should suffice but in chronic cases prolonged treatment is necessary. In pyelitis, usually there is mixed infection and elimination of sensitive organisms reduces toxæmia.

Perinephric abscess and carbuncle of the kidney have been successfully treated with penicillin, the usual dose given being 100,000 units or more 2 or 3 times a day. Single and multiple pyogenic infections of the renal pelvis and urinary bladder have responded well to penicillin treatment.

In salpingitis due to sensitive organism, massive doses lead to absorption of the exudate without obliteration of the lumen of the tubes. In pelvic cellulitis, penicillin therapy should be immediately started, three doses of 100,000 to 200,000 units being given in the first 24 hours followed by 50,000 units every 6 to 8 hours for 2 or 3 days.

Puerperal Sepsis, Breast abscess, etc.—Provision of an adequate blood level during puerperium and delivery acts as prophylactio in cases of difficult labour where operative procedure is necessary. For this purpose 100,000 units or more may be given daily for 2 or 3 days. When actual infection has occured, an initial dose of 500,000 units followed by adequate doses to maintain a steady level are best. In hospitals, the drip method should be employed. When septicæmia has occured 100,000 to 200,000 units twice daily give good results.

Local application of penicillin in form of cream is effective in the treatment of cracked nipple. In breast abscess, systemic use combined with local use is best. In incipient abscess, 100,000 to 200,000 units twice daily for two days often abort abscess formation.

(8) Venereal infections

There is no doubt that penicillin is most useful in the treatment of venereal diseases.

Gonorrhoea is most easily treated with penicillin. Strains of gonococci often become resistant to the action of sulphonamides even when combined with fever therapy, and therefore are not eradicated. Such patients can be successfully treated with penicillin. Striking changes for the better occur in the urethral discharge within 3 to 4 hours after commencement of penicillin treatment, viable gonococci disappearing.

Gonorrhoea in the male.

In the treatment of gonorrhoea in ambulant patients of either sex, the method of choice is the administration of a single dose of procaine penicillin, 300,000 units. Alternatively a total of 100,000 to 150,000 units of crystalline penicillin in five equal doses at intervals of two hours may be given. Smaller doses are also effective but larger doses are advisable. With such treatment the cure rate is more than 98 per cent. If prostatitis and epididymitis are present, these should also be treated with penicillin otherwise cure of the infection may be prevented. It should be remembered that about 3 per cent of cases of acute gonorrhoeal infections are likely to be incubating syphilitic infection and there is possibility of symptoms being markedly modified or delayed if penicillin is given. This, however, is not common as most of the combined cases develop the primary sore within 60 days.

Gonorrhoea female.

In gonorrhœa in the female, such complications as pelvic inflammation, salpingitis, gonorrhœal proctitis and vulvo-vaginitis, yield to penicillin. In salpingitis massive doses are given over a period of three or four days. In vulvo-vaginitis in children 100,000 units or more daily for a few days are effective.

Penicillin suspensions in oil and beeswax and procaine penicillin are largely used with excellent results and one single injection of 300,000 units has produced equally good results as 100,000 units in aqueous solution in five equal doses. A proportion of failures and relapses occur and many of these respond to a second course. Some, however, require an intensive treatment with increased dosage even up to 1,000,000 units continued perhaps with sulphonamide and fever therapy.

Test for cure.—The patient should be watched for at least 12 weeks and during this period the urine and prostatovesicular secretion should be microscopically and culturally examined. A persisting urethritis and cervicitis is very suspicious even when gonococci are not found, and the patient should not be declared free until he is symptomless for 3 months.

Gonorrhoeal arthritis.—In gonorrhoeal arthritis doses of 100,000 to 200,000 units are given twice daily. When gonorrroeal arthritis complicates gonorrhœal urethritis, longer and more intensive treatment is necessary, till clinical improvement is evident and negative cultures are obtained. Intra-articular instillation is not necessary in case of small joints but in case of large joints 50,000 units or more in 10 ccm of normal saline should be injected after aspiration, every 24 to 48 hours till three or more injections are given.

Gonorrhœal endocarditis is generally a fatal complication as by the time it is recognized, infected vegetations have developed. Chances of cure have however considerably improved with modern methods of intensive penicillin therapy, *i.e.*, 10 to 12 mega units daily continued for at least 8 weeks.

(9) Syphilis and other spirochætal infections

In the treatment of syphilis, the present view is to combine penicillin with arsenicals. A scheme suggested is to give 4,800,000 units of penicillin in seven and a half days with ten daily injections of 0.09 gm (1½ gr) of neohalarsine or 0.06 gm (1 gr) of mapharside. Toxic effects are rare but if these occur arsenic is stopped and penicillin is increased to 4,000,000 units. Relapses are treated with four million units of penicillin and ten daily injections of arsenoxide. If penicillin is combined with 0.6 to 1.0 gm (10 to 15 gr) of bismuth the relapse rate is even lower. The efficacy of treatment depends on early commencement, the chances of cure decrease with duration of infection and in late cases much larger doses have to be given. An ambulatory treatment suggested by Llyod

Jones and Maitland consists in giving a single injection of 300,000 units daily for thirteen days, or 500,000 units daily for ten days in primary syphilis. In secondary syphilis 300,000 units are given daily for sixteen days or 500,000 units daily for ten days.

All early cases of syphilis must be observed for at least 2 and preferably 5 years after treatment. At least three serological tests should be made during the first 6 months and a further 6 at three monthly intervals for 2 years. A full examination of cerebro-spinal fluid should be made after 6 and 24 months, and skin and mucous membranes examined for signs of muco-cutaneous relapse. Clinical relapse may occur before the blood tests become positive. Sero-positive cases may take six months to revert to negative but falling or rising titres are helpful indications.

Neurosyphilis.

After the first few injections of penicillin, there may be malaise or headache, and primary sore and secondary symptoms may become pronounced. General urticaria and in a few cases severe systemic reactions may appear.

Syphilis in pregnancy.

In neurosyphilis there is undoubted clinical improvement and penicillin therapy often is combined with malaria therapy or routine chemotherapy. A total dosage of 4,800,000 units in seven and a half days is suggested. In pregnancy complicating syphilis, treatment is started with small doses which are gradually increased to reduce possibility of abortion. A total dosage 1,200,000 to 2,400,000 is given but larger doses are preferable, spread over a period of 8 to 9 days. In congenital syphilis 20,000 to 40,000 units are given three hourly but larger doses exceeding 20,000 per pound body weight are suggested for 10 to 15 days.

Herxheimers reaction.

Reactions resembling that of Herxheimer's occur in more than 50 per cent of cases during the first 24 hours of treatment. There may be fever alone or exacerbation of local lesions with or without fever. Symptoms are rarely alarming and penicillin can be continued. In a few cases, skin rash and fever may occur. Therapeutic shock (Herxheimer's reaction) may occur in early or late syphilis; in case of late syphilis the reaction is severe and may be serious. Severe cerebro-spinal symptoms may appear in neurosyphilis under penicillin treatment. Reduced doses of penicillin given in first 24 or 48 hours may prevent severe reactions.

Prophylactic use against syphilis.—This question has not been properly worked out nor can it be definitely said whether an intensive course of penicillin therapy within the first day or two of exposure to syphilis can prevent the disease.

Other spirochaetal diseases.

Penicillin has also been tried in the treatment of yaws, relapsing fever and Weil's disease, with good results.

(10) Miscellaneous infections

Wounds.—If wounds are small, superficial and clean, dust with penicillin-sulphathiazole powder. Septic wounds are treated with powder and cream for a day or two before they are sutured. For deep wounds, pending operation, apply cream on sterile gauze. If wounds are deep and penetrating, penicillin applied locally is not effective.

Experience during World War II has shown that in case of ordinary wounds, application of powder is effective, but in severe and obviously contaminated wounds both local and systemic treatment is necessary, 50,000 units being given intramuscularly every three hours or 200,000 units or more by the continuous

method. Parenteral treatment is seldom necessary for more than three days and is discontinued generally after 48 hours if the wound is painless and otherwise quiet. In severely lacerated and contaminated wounds and those which have been sutured under tension, penicillin is continued for 4-5 days and stitches should not be opened for 11-12 days. If anærobic infection is present, double the amount of penicillin should be given. Penicillin therapy with sound surgery is essential for success.

Gas Gangrene.—Infection with *Cl. welchii* is successfully treated with penicillin by local and parenteral application. Penicillin, however, does not counteract the toxins; all necrosed muscle and other damaged tissue should be removed, and antitoxin should be administered as soon as the presence of the disease is suspected. Penicillin should be applied locally in the form of powder of penicillin and sulphathiazole (500 units per gm) or in physiologic saline solution containing at least 1,000 units per ccm; it may also be instilled into the wound by means of tubes, three or four times a day. In addition, penicillin should be given in doses of 100,000 to 500,000 units by intramuscular or intraveous drip method till satisfactory improvement is apparent. Local or systemic use of penicillin does not prevent development of gas gangrene.

Anthrax.—Excellent results have been obtained in the treatment of infections with *B. anthrax.* An initial dose of 500,000 units should be followed by doses of 100,000 units every three or four hours.

Actinomycosis.—Nearly all strains of *Actinomyces bovis* are fairly sensitive to penicillin. Infection of bones such as the maxilla has been successfully treated with penicillin as also abdominal actinomycosis. After surgical drainage at least 200,000 to 300,000 units and in some cases more should be given twice daily for three weeks and the course may have to be repeated. After evacuation of pus, penicillin solution (1,000 units per ccm) is instilled into the cavity. Between the courses a rest period of 2 to 4 weeks should be allowed.

Virus Infections.—It is known that penicillin is effective in the treatment of ornithosis and psittacosis and such infections in man have been successfully treated with 100,000 units a day continued for 3 to 7 days.

(11) Penicillin in veterinary practice

Assays of urine in cows after intramuscular administration 300,000 units of penicillin every 4 hours for five days or till 10,000,000 are given showed that adequate concentrations existed to affect *Cor. renale* the causative organism of pyelonephritis. Penicillin can, therefore, be used in bovine nephritis and also other conditions. In the milk it only occurs in negligible quantities. Penicillin concentration in the udder depends in part on the extent and rate of absorption or loss and also on dilution by the secretion of milk. Intramammary injection is, therefore, best.

Bovine mastitis produced by *Str. agalactiae* which is sensitive to penicillin is easily controlled. Mastitis produced by *Staph. aureus, Str. dysgalactiae* and *Str. urberis* also react to penicillin but not so well. Systemic treatment is not effective and local injections of penicillin in sterile aqueous solution through the teat canal into each quarter of the udder with strict aseptic precautions are useful; 20,000 units dissolved in 50 ccm of distilled water are injected into each affected quarter.

In horses penicillin is used in the treatment of strangles, an acute infection producing inflammation of nasal and pharyngeal mucosa with suppuration of

regional lymphatic glands due to *Str. equi;* 5 to 9 injections of 100,000 units are given at intervals of 3 to 4 hours. Swine erysipelas caused by *Ery. rhusiopathiae* is amenable to penicillin therapy.

(12) Prophylactic uses

Now that penicillin is available in large quantities it is being used as a prophylactic before surgical and dental operations. There is also likelihood of using it on a large scale as an aerosol in operating theatres, hospital wards, cinemas, factories, etc.

Uses in wounds.

It should also be used as a prophylactic in all potentially infected wounds. The minor and more superficial wounds only need dusting with penicillin powder 5,000 to 15,000 units per gm of sulphathiazole, sulphamethazine or powdered plaster. In civil practice where wounds are attended to quickly, 500 units per gm are effective. In severe and contaminated wounds, both parenteral administration (100,000 to 120,000 units) and local application are necessary. In badly infected wounds larger quantities are given, the initial dose being 300,000 units.

Penicillin was used during the World War II as a prophylactic in case of wounds. Most of the dangerous organisms found in war wounds are fortunately penicillin sensitive and here is every prospect of their being inhibited or being destroyed with adequate concentrations of this drug. It was applied locally in minor wounds and in major wounds after adequate primary surgery, penicillin powder (5,000 units in 1.0 gm of sulphonamide) was applied to the wound. Later 100,000 units were given as soon as circumstances permitted, followed by injections of 50,000 units at five-hour intervals.

Penicillin versus sulphonamide.—The experience during the World War II showed that penicillin was a more effective agent than sulphonamides in routine prophylaxis in doses of 5.0 gm daily for five days. On an analysis of cases treated the number of severe infections was highest when sulphonamides only were given; in the groups which had penicillin only and those who had sulphonamides and penicillin, there was no difference, showing that sulphonamide did not help. The conclusion drawn from these results is that parenteral administration in addition to local use of penicillin is essential in severe war wounds to ensure maximal protection.

Airborne infections.

It has been suggested that penicillin is of value in form of lozenges or sprays in preventing airborne infections and in limiting epidemics of diphtheria and other sensitive pathogenic organisms. It may be used as a prophylactic against venereal infection, but there is the possibility of producing resistant strains as with sulphonamides. In case of penicillin, relative resistance develops but organisms originally sensitive to penicillin have not been shown to have become insensitive.

(13) Conditions susceptible and insusceptible to penicillin

The conditions amenable to treatment with penicillin are:—

(1) Septic wounds, arthritis, abscesses, carbuncles, burns, acute and chronic rhinitis, sinusitis, acute and chronic recurrent tonsillitis, acute and chronic pharyngitis, quinsy, etc.—Both local and systemic administration are used.

(2) Lobar pneumonia, broncho-pneumonia, acute and chronic recurrent bronchitis, bronchiectasis, pleural effusions and empyemata.—local inhalations and systemic therapy.

(3) Acute abdominal conditions, *e.g.*, peritonitis, appendicitis, chlolecystitis, etc. chiefly by systemic therapy.

Susceptible to penicillin.

(4) Meningitis, otitis media, mastoiditis, sinus thrombosis, wounds of brain and its meninges, bone infections, acute and chronic osteomyelitis.—Systemic therapy in large doses and local use.

(5) Genito-urinary infections, puerperal sepsis, urethritis and arthritis (gonococcal).—Local and systemic therapy.

(6) Syphilis in all stages, neurosyphilis, yaws, leptospirosis.—Systemic therapy in massive doses.

(7) Generalised blood infections such as acute and subacute bacterial endocarditis, streptococcal and staphylococcal septicaemias.—Systemic therapy in massive doses.

Penicillin in septic conditions.

As prophylactic, penicillin is used in all operations, major or minor when there is danger of spread of sepsis. The tendency now is to give massive doses, a single dose of 300,000 units or 100,000 units twice daily. The development of oil-beeswax combination and procaine penicillin will make it possible for only one or two injections daily to deal effectively with many conditions. For local applications, solutions of penicillin, creams, ointments, sprays, drops, snuffs, powders, lozenges, and suppositories, etc., are used.

Penicillin therapy should be combined with surgery where collections of pus, septic fluids, etc., are present or there are other indications. In case of serious septic conditions, *e.g.*, carbuncles, abscesses, etc., a penicillin barrier may be established by injecting round the focus.

It should be borne in mind that susceptible bacteria may become resistant to penicillin during treatment. It should also be realised that there is no correlation between resistance to penicillin and resistance to sulphonamides. Organisms which become resistant to the former are not resistant to latter; the converse is also true. The two drugs can be used together as adjuvants to each other. Every use should be made of these two drugs in the prophylaxis and treatment of infections in a carefully considered manner.

Insusceptible to penicillin.

The following conditions are considered to be insusceptible to penicillin at present:—Typhoid, paratyphoid, dysenteric infection, cholera, pneumonia due to Friedlander's bacillus, influenza, brucellosis, virus infections, pasteurella infections (plague, tularaemia), tuberculosis, infection with *E. coli* organism, yeast and mould infections.

11. Methods of Assay and Standardization

Oxford workers adopted a purely arbitratory unit defined as "that amount of penicillin contained in one millilitre of a certain phosphate buffer solution containing ether". This is the original Oxford Unit. Later the unit was defined as "the amount of penicillin which when dissolved in one millilitre of water gave the same inhibition as a certain standard. This standard was a "partially purified solution of a purely arbitrary strength, made up in dilute phosphate buffer, is saturated with ether and kept in ice-chest". Later it was defined as "that amount of penicillin which when dissolved in fifty millilitres of meat extract just inhibits completely the growth of the test strain of *Staph. aureus*". None of these definitions are satisfactory for general use and most laboratories relied on their own standards.

Provisional British Stndard (P.B.S). This was evolved by the National Institute for Medical Research till establishment of an international unit. On this basis the potency assigned to it was 155 units per milligram. In America a *"master standard"* was prepared by mixing a number of highly purified erystalline products and this was compared with Provisional British Standard. In general the potency of P.B.S was found to be somewhat higher than the accepted figure of 155.

The International Standard was worked out by assaying a large number of samples, British and American as compared with U.S. Master Standard, which was prepared from crystalline sodium penicillin II. (G). The international unit was defined as the *specific activity contained in* 0.6 *microgram of the International Penicillin Standard,* and is approximately equivalent to the Oxford Unit. Its purpose is to provide a check on the various national standards, and should not be used for routine assays. The international working standard consists of a sample of calcium penicillin with a potency of 370 units per mgm. This was dried and sealed in tubes containing 65 mgm. One international unit is contained in 2.7 micrograms of the international standard unit. *International unit.*

British National Standard was worked out by taking 30 gm of crystalline sodium penicillin II divided into 30 mgm sealed containers. Samples of these were assayed by six different laboratories, against the international standard. Activity contained in 0.625 microgram of British National Standard is equivalent to one international unit. This standard preparation has been adopted for purposes of regulations made under Therapeutic Substances Act, 1925. The unit has been defined as the activity contained in such an amount of the standard preparation as may be indicated by the Research Council for international use. The B. P. method requires potency of penicillin to be determined by comparing the quantity which inhibits the growth of a sensitive strain of *Staph. aureus* with the quantity of standard that produces the same inhibition. Details are given in the Pharmacopoeia.

The U.S.A. Method, adopted by the Food and Drug Administration is similar but is described in more detail.

Determining concentration in body fluids

All methods for determining the concentration of penicillin in various body fluids are based on tests which measure the degree of bacteriostasis produced by the sample under assay.

Two methods are generally described for assay: (1) by measuring the distance through which the test organism is inhibited on an agar plate by penicillin solution (2) by observing the dilution of penicillin solution which completely inhibits the growth of the test organism in fluid culture.

Serial dilution method.—The oxford unit was originally defined as the amount of penicillin which when dissolved in 50 ccm of meat extract broth, would just inhibit completely the growth of test strain of staphylococci. To determine the activity of a solution of unknown strength, dilutions of this and of the standard solution are made in broth and each is inoculated with the test staphylococci. These are then incubated overnight and the dilution which completely inhibits growth is noted in each case. In modification of serial dilution by Rammelkamp a highly sensitive strain of B-hæmolytic streptococcus is used as a test organism and red blood cells as indicator. This method is probably more easily adapted to routine clinical practice. The other method is to note inhibition of test organism on an agar plate by penicillin. Rapid methods of assay are necessary during manufacture and for this a turbidimetric method using photo-electric apparatus has been evolved and a curve of turbidity is obtained for broth inoculated with staphylococci containing known amounts of penicillin. With this the unknown strength solution is compared. *Oxford unit.*

Methods have also been devised for assay of creams, ointments, dusting powders, snuffs, tablets, lozenges, oil and beeswax suspensions.

Micro methods.—These are specially useful in assay of blood of the patient when his condition justifies the slightest interference. It can be carried out with one drop of blood. Other methods such as *Slide-cell Method* and *Capillary tube method* have been developed.

Chemical assay.—This is based on inactivation of penicillin by penicillinase leading to the liberation of penicilloic acid which can be directly titrated. The method is said to give satisfactory results.

Colorimetric method.—A coloured component is formed by interaction of penicillin and N-(1-naphthyl-4-azobenzene) ethylenediamine and a method of colorimetric determination based on this reaction has been evolved.

Estimation of penicillin sensitivity of infecting organisms

This is of practical importance in penicillin therapy. These methods also fall under serial dilution method or measuring the distance to which a culture is inhibited on an agar plate. In both cases exact measurements are required with control of a standard culture of staphylococci under exactly the same conditions.

The oxford cup method.

Other methods in use are: *Quick assay method of Rake and Jones* in which hæmolysis is used as an indicator; *The Oxford cup method* which is based on the fact that if penicillin is allowed to diffuse into the medium of a plate previously seeded with test organism, a zone of inhibition around the point of diffusion occurs; the diameter of this zone of inhibition is taken as a measure of concentration of penicillin; The *tissue culture method* in which pneumococcus is used as a test organism and which grows on the tissue culture medium.

Of the methods devised for determining the presence of penicillin in the body fluids, the methods of Flemming and Rammelkamp are commonly used. Such methods, however, are not very accurate but give an idea of the penicillin level in the blood and its bacteriostatic activity.

12. Pharmaceutical Aspects of Penicillin

Dispensing of penicillin.

The dispensing of penicillin presents new problems. All the aseptic precautions necessary for preparation of an injection have to be taken. Contamination with penicillinase producing organisms should be avoided. The difficulties of sterilization are increased because the sterile product has to be prepared from day to day and injections have to be given frequently. Moisture has to be excluded; pH should be within range of 5 to 8; temperature cannot be raised; contact with heavy metals (Pb, Cu, Hg, Zn, etc.) is deleterious as also with alcohol, glycerine and oxidising agents.

Bacteriological cleanliness.

Bacteriological cleanliness is essential and preparations for application to wounds should be sterile. U S. Food and Drug Administration laws require that in case of such preparations, as ointments, snuffs, etc., which need not be sterile, the content of viable microorganisms is not greater than is consistent with good pharmaceutical manufacturing practice.

Penicillin is included in B. P. and is defined as the anti-infective acid produced by *P. notatum* or related organisms grown under appropriate conditions on or in a suitable culture medium, converted into sodium or calcium salt. The term penicillin is applied to both sodium and calcium salts. It is controlled by Therapeutic Substances Act with regard to conditions of manufacture, assay, standards of purity, packing, labelling, storage, sale, etc. It should pass tests of freedom from abnormal toxicity, freedom from pyrogens and tests for sterility, etc. The sealed containers should have labels indicating whether it contains the sodium or calcium salt and the total number of units per mgm of penicillin. The salts are issued in rubber capped vials each containing 100,000, 200,000, 500,000 or 1,000,000 units of salt. One million units equal one mega unit.

Potency of penicillin.

According to B. P. penicillin salts must have a potency of not less than 300 units per mgm. The samples on the market have a potency varying from 300 to 1,400 units per mgm. Pure sodium penicillin can be obtained as white powder. In pure form these salts may have a potency of as much as 1,666 units per mgm.

The reason why penicillin of lower potency is not allowed for parenteral injection is that it gives rise to such toxic reactions as urticaria and fever on account of impurities present. The B. P. requires that penicillin for parenteral injections should have a potency of at least 900 units per mgm. Preparations with lower unitage are used for making preparations for local use, *e.g.,* creams, powders, lozenges, etc.

It has already been stated that penicillin has a powerful bactericidal action and its activity is not hindered by the presence of body fluids, blood, serum, pus, etc. It is excreted in the urine rapidly and for this reason its concentration in the blood falls. Repeated doses have to be given to maintain an effective concentration in the blood. If the solution has to be kept for repeated injections, chlorocresol is added as an antiseptic, but it is not free from deleterious effects. Solutions for intrathecal injection should be free from chlorocresol if the volume for injection is to exceed 15 ccm. No aqueous solution should be stored for more than seven days even at a temperature of 4°C.

Stability.

Penicillin is rapidly destroyed in presence of moisture. Its sodium salt is hygroscopic and is liable to decomposition under storage. It is more suited for making aqueous solutions while the calcium salt is suitable for dry preparation such as tablets, powders, etc. The watery solutions are unstable but dry powders and oily suspensions do not lose their activity. Penicillin is rapidly destroyed in the presence of acids and alkalies. The optimum stability of penicillin is at pH of 5.5 to 7.5. Heavy metalic ions such as silver, mercury, copper, lead, etc., destroy it in aqueous solutions but not in oily or fatty suspensions.

The enzyme penicillinase which may sometime be present in non-sterile distilled water, rapidly destroys it. This enzyme can be destroyed by boiling water for 10 minutes. The specific action of penicillin is only on the gram-positive bacteria and sometime these develop resistance to it. This occurs under clinical conditions when there is under dosage.

Suspensions of dry calcium penicillin in oil or in fatty medium are as stable as the powder, and retain full potency for six months at room temperature. Rancidity in vegetable oils renders it inactive. Penicillin in glucose solutions should not be heated as the pH is likely to fall to 4.0 and it will become inactivated.

Briefly it may be noted that the contact of penicillin with the following is likely to lead to deterioration and should be avoided.

(1) Moisture. (2) The optimal stability is at pH 6 and reaction below 5 and above 8. (3) Temperatures above 10°C. (4) Contact with heavy metals (Cu, Hg., Zn, Su), Alcohol, Glucose, oxidising agents and penicillinase.

Freedom from bacteria is essential for ointments, creams, etc., to be applied to infected wounds. Although strict asepsis is not necessary for such preparations as ointments to be applied to the skin, lozenges, snuff, etc., sterility is essential to exclude penicillinase producing organisms. Antiseptics if used may produce deterioration. The point of practical importance is that although some of these preparations need not be absolutely sterile they should be prepared under conditions of utmost cleanliness so that they are not grossly contaminated.

Storage.

In dry conditions (in tablets and powders), penicillin maintains its activity for a long time when stored in sealed tubes at temperatures below 15°C. Penetration of moisture leads to its deterioration and should be rigidly excluded. Stock solutions should be stored in refrigerators at 4°C. Phosphate buffers have to be used to prevent pH from changing in creams, etc., but not in aqueous solutions for parenteral use. Tablets are more stable than powders but should be stored at temperatures not exceeding 15°C. Stability increases with the degree of purity and pure sodium salt in dry condition is stable at 100°C.

Compatibility.—In dry form penicillin can be mixed with sulphonamides and made into powders, tablets, etc. It is not inactivated by certain antiseptics, *e.g.,* chlorocresol 0.1 per cent, chlorobutol, iodized oils, thymol, menthol, etc. It is also compatible with alkaloidal salts, such as of ephedrine and adrenaline, but not in acid solutions. It is incompatible with R-S-H group such as cysteine or thioglycollates, metallic ions, methyl alcohol, ethyl alcohol (except in concentrations below 25 per cent). It is said to be less stable in glycerine than in water..

Administration.—On account of its rapid excretion attempts have been made to combine penicillin with substances which will prolong its stay in the blood by mixing it with benzoic acid or p.-aminohippuric acid. Suspensions of calcium salts in arachis oil, beeswax, etc., have been used. Preparations containing calcium penicillin and ethyl oleate have been tried and it was found that though suspension in arachis oil gave a lower concentration in blood it was maintained for a longer period than ethyl oleate.

Aqueous solutions are prepared in a sterile isotonic solution of sodium chloride and occasionally in sterile isotonic solution of glucose (care should be taken to see that it has not turned acid). In the B.P. two solutions have been included for parenteral use; one in normal saline and the other a suspension in oil.

Injectio penicillini B.P.

Injectio penicillini B.P. for intramuscular, intravenous and intrathecal use, is prepared by dissolving the contents of sealed container (sodium or calcium salt of not less than 900 units per mgm) in pyrogen free physiological saline. When no strength is prescribed one containing 20,000 units per ccm is dispensed; 0.1 per cent chlorocresol is put in as bacteriostatic if several doses are required in the same container but it should not be added to solutions for intrathecal use. Nor should it be added if the volume of doses is more than 15 ccm. No aqueous solution should be stored for more than 7 days at temperature of 40°C. All aseptic precautions should be taken.

In place of sodium chloride solution 5 per cent W/V (isotonic) glucose solution may be used; this is said to give a more prolonged action. Solutions containing 5,000 *i. u.* in 4 ccm of 1 in 50,000 adrenaline are also used for delaying absorption.

Although the B.P. lays down storage for injections at 4°C this is not necessary as storage at room temperature for a week produces no significant deterioration.

Injectio penicillini oleosa (*B.P.*) for intramuscular use contains 125,000 units per ccm. This preparation is viscous and should be warmed to blood heat before injection but too much heat is undesirable. The syringe to be used is also warmed and both the syringe and needle should be perfectly dry before use. If stored in a cool place this preparation should retain its potency for six months. There is demand for more potent (200,000 to 300,000 *i. u.* per ccm) suspensions but these are even more viscous than the B. P. injection. For such preparations calcium penicillin of highest potency available should be used. The U.S. Food and Drug Administration recognise three strengths namely 100,000 200,000 and

300,000 i.u. per ccm. It is also stated that storage should be at 15°C, whereas B. P. requires merely "in a cool place". It should be borne in mind that beeswax is not suitable for injection in quantities larger than 0.2 gm (3 gr.), the amount contained in the maximum dose of the B. P. preparation. The content of beeswax should not be less than 3 per cent.

3. *Ethyloleate suspensions* have been prepared with a view to obtaining greater fluidity and possibly avoiding the need for warming before use, arachis oil being used as a vehicle. Other vegetable oils such as olive oil have also been suggested. Ethyloleate suspensions are said to give less prolonged bacteriostatic concentrations in the blood than suspensions of penicillin in arachis oil.

4. *Suspensions of penicillin in iodised oil* have been proposed for introduction into the lung in bronchiectasis. These are prepared in the same way as injectio penicillini oleosa B. P. and are said to be stable even at 37°C for more than 2 months. The hygroscopic properties of sodium penicillin have been counteracted by incorporating partly dried magnesium sulphate.

5. *Emulsions.* Emulsion in an aqueous solution in an oil base has been suggested as an alternative for suspension in oil and beeswax. The penicillin is dissolved in physiological saline and gradually added to sterile arachis oil mixture, shaking vigorously to form an emulsion.

Oral penicillin.

6. *Preparations for oral use.* (*a*) Capsules and enteric coated capsules have been prepared, 100,000 *i.u.* powders being put in a gelatine capsule which is sealed and enclosed in the next larger size capsule. The whole is immersed in 1 in 20 solutions of formaldehyde (40 per cent) for five seconds and next in 95 per cent alcohol for five minutes. These are given with two tablets of aluminium hydroxide as an additional precaution.

(*b*) *Mixture.* Penicillin in mixture form with sodium citrate is convenient and reasonably stable means of giving penicillin by the mouth for systemic effects. The following mixture has been suggested: Sodium penicillin 400,000 *i.u.*, sodium citrate 16 gm (½ oz), syrup 30 ccm (1 oz.), chloroform water to 250 ccm (8 oz), each ounce of this mixture contains 50,000 *i.u.* of penicillin. The mixture is given every two hours during the day and every four hours at night. Another combination is to use, aluminium hydroxide gel 120 ccm (4 oz), and 400,000 *i.u.* of sodium penicillin and make up with distilled water to 250 ccm (8 oz). A mixture with magnesium hydroxide is prepared in the same way. The U. S. Food and Drug Administration requires that penicillin and aluminium hydroxide gel be kept in separate containers.

(*c*) *Powders.* In America powders are used for oral administration. Calcium penicillin 50,000 *i.u.*, sulphanilamide 1.0 gm (15 gr), sodium bicarbonate 1.0 gm (15 gr), sodium citrate 2 gm (30 gr), oil of pepperment one drop. Each powder is prepared separately and is put in glass tube. Before administration the powder is stirred in water. Sulphanilamide may be replaced by sulphathiazole and sulphadiazine, etc.

Local use.

(7) *Preparations for local use* (a) *Dusting powders.* (b) *Tablets.* Tablets of the sodium, calcium, or potassium salts, either plain or bufferred are now available in the market. They are usually dispensed in strengths of 25,000 50,000 or 100,000 units per tablet. The following are some of the examples:—Calcium penicillin 60,000 to 150,000 *i.u.*,, finely powdered sulphathiazole 30 gm (1 oz). Sulphathiazole is heated to 100°C to remove moisture and then heated in a hot air oven at 150°C for one hour. Other examples are calcium penicilln 150,000 *i.u.*, sulphanilamide 1-5 gm (24 gr), light magnesium oxide 28.5 gm (1 oz)-(2) Penicillin 120,000 *i.u.*, dried human serum or plasma 30 gm (1 oz), or calcium penicillin 60,000 i.u., lactose 30 gm (1 oz). Lactose is dried at 100°C then sterilised at 150°C for one hour.

For storage of these powders a low temperature is desirable but no deterioration takes place for a month or two at room temperature. Sterilised glass stoppered bottles or screw-capped glass jars are used for storing.

Eye drops.

(b) *Eye drops.* These are prepared in strengths of 2,500 i.u. per ccm, but those of 10,000 *i.u.* per ccm are also used. Addition of 0.5 per cent chlorocresol has no irritant effect. Examples are, sodium penicillin 75,000 i.u., chlorocresol 0.015 gm (1/5 gr), sodium chloride 0.42 gm (½ gr), distilled water 30 ccm (1 oz). Sometimes *carbowax* 900 is added to prolong action by making the solution more viscid.

Nasal drops.

(c) *Nasal drops.* These are prepared with physiological saline with 0.1 per cent chlorocresol. The composition is the same as eye drops.

(d) *Lozenges. Trochiscus Penicillini B.P.* contains 500 i.u. of calcium penicillin but American manufacturers supply lozenges with 1,000 i.u., in each. A properly made lozenge should remain in the mouth for 40 minutes and this makes the preparation difficult. Lozenges should be kept in the buccal sulcus for 24 hours except for meal intervals; as soon as one is finished another one is put in. One lozenge usually lasts the whole night due to decreased activity in buccal cavity during sleep. This treatment is likely to cause development of sores in the mouth and tongue which may necessitate suspension of treatment. Only occasional sucking of lozenges for treatment is very undesirable for obvious reasons as the object is to apply constant and maintained antibacterial concentrations of penicillin. *Trochiscus penicillini B.P.*

Properly prepared lozenges retain better potency at room temperatures for more than six months if moisture is excluded.

(e) *Pastilles.* These are not anhydrous and therefore less stable. They also do not last as long in the mouth as lozenges, seven minutes being the maximum period.
Pastilles can be prepared with gelatine or agar base.

(f) *Ointments, Creams, etc.* It was at one time considered that they should be sterile, but it is now agreed that this is not necessary as long as provisions are made to prevent multiplication of penicillinase producing bacteria. This purpose is served by putting in such as antiseptic as chlorocresol which is effective against a wide range of bacteria. This has now been accepted by British Pharmacopoeia Commission.

Cremor Penicillini Sterilisatus B.P. and Cremor Penicillini B.P. are both made with lanette wax SX and liquid paraffin. The ointment is said to be stable at room temperature for 6 to 9 months. It should be kept in a cool place in a well-closed container to protect it from moisture.

The ointment is suitable for all superficial skin infections and minor wounds. It should not be used for acne for which ointments with a non-fatty base are preferred. The potency should not be less than 250 i.u. per gramme. Buffered bases are used to overcome increase in acidity. Liquid paraffin, castor oil, cetyl alcohol, water-in-oil and stearate bases are also used. For non-greasy bases, agar, tragacanth, jelly, etc. have been used.

Oculentum Penicillin B.P. has the same strength as the ordinary ointment.

(g) *Lotions* are used for medicating dressings or application as compress on gauze in burns or skin infections. The strength is 250 to 1,000 i.u. or more per ccm the usual solvent employed being physiological saline, but for many conditions sterile water is preferable on account of the saline leaving a deposit of sodium chloride crystals on skin wounds. Solutions should be sterile and are generally freshly prepared; 0.1 per cent of chlorocresol is added to prevent development of penicillinase-producing organisms. *Lotions.*

(h) *Sprays.* Sprays are a convenient method of applying lotions to superficial areas. Very fine sprays or "aerosols" are inhaled with the object of carrying penicillin into bronchioles and alveoli of the lung and for this strengths of 20,000 to 100,000 i.u. per ccm are suitable. The size of particle of the spray should be small. To test this, spray from a nebuliser is directed against a glazed surface; no visible deposit should form. Glycerine 5 to 20 per cent is included in the spray as it is said to render the preparation less irritating, though it may slightly effect its stability. The spray should be sterile. A dosimetric spray has been designed for the patient to know the exact volume of solution nebulised and inhaled. *Sprays.*

It has been shown that 20 ccm of solution of sodium penicillin containing 1,000 i.u. per ccm will give a therapeutically effective concentration in the blood, when vaporised in a room of 3,000 cu. ft. capacity. This shows the possibilities of this method for systemic treatment of disease. Addition of adrenaline, or ephedrine hydrochloride to sprays according to some authorities facilitates action of penicillin. As much as 60 per cent of penicillin inhaled through sprays appears in urine. Examples of sprays are:—(a) Solution of adrenaline hydrochloride 2 ccm (30 minims), Calcium penicillin 1 mega unit, distilled water 100 ccm (3½ oz). (b) Calcium penicillin 30,000 *i.u.*, ephedrine hydrochloride 0.15 gram (2½ grains), physiological saline 30 ccm (1 ounce).

Snuffs.—Snuffs are used for treatment of nasal infections and they need not be sterile. Following are examples: (a) Calcium penicillin 30,000 i.u., blood plasma or glucose 30 gm, (b) Calcium penicillin 0.3 gm (300,000 i.u.—5 grains) menthol 1.5 gm (25 grains), lycopodium 30 gm (1 oz), (c) Calcium penicillin 300,000 i.u., sulphathiazole 30 gm (1 oz). Such snuffs taken in doses of 0.1 gm half in each nostril are effective. Snuff is administered by putting the powder on a paper bringing it near one nostril closing the other nostril accompanied by a sharp sniff, the head being kept tilted. An effective concentration is maintained in oropharyngeal secretions provided no food or drink is taken.

Lamellae.—These are made with gelatine base each containing 100 to 250 i.u.

Suppositories.—Rate of absorption of penicillin from rectum exceeds the rate of destruction and it is possible to obtain therapeutic concentration in blood by this method. Calcium penicillin 0.3 to 1 mega unit, oil of theobroma 1 gm (15 gr).

Pessaries.—These have a gelatine base and are used for erosion of cervix or infections of vagina.

13. Penicillin and Sulphonamides

Penicillin does not appear to be absorbed or destroyed during bacterial inhibition. Unlike sulphonamides the action of penicillin is not inhibited in the presence of pus or products of breakdown of tissues or para-amino benzoic acid. Difficulty often arises in choosing between sulphonamides and penicillin. Penicillin in addition to being non-toxic is generally more potent in vitro, preventing growth of organisms in dilution of one in one hundred million. Its action is also not affected by tissue lysates in pus, necrotic tissue or blood, and it is active in the presence of large inocula of bacteria. Its disadvantages are that it cannot at present be given by the mouth with certainty of absorption, it has to be administered by frequent injections or by drip method, it deteriorates in presence of heat and moisture and it is destroyed in the presence of certain bacteria and enzymes.

It is well known that a number of micro-organisms are sensitive to penicillin, but are not acted upon by sulphonamides (syphilis, yaws, gas gangrene, etc.). Coli-typhoid dysentery group, Friedlander's bacillus, *H. influenzae,* Ducrey's bacilus, Brucellæ and Pasturellae are acted upon more by sulphonamides but not penicillin and difficulty does not arise as to which should be used.

Str. viridans, haemolytic streptococcus, staphylococcus, meningococcus, gonococcus, clostridia (welchii, septicum and tetani), *B. anthracis* and actinomyces are acted upon both by penicillin and sulphonamides. It may be stated in general that in those infections in which bacteraemia, suppuration and necrosis are present penicillin should be preferred, if these are not present sulphonamides may be used. Penicillin is preferable in severe cases of puerperal sepsis, cellulitis, mastoiditis, endocarditis, pericarditis, peritonitis, suppurative arthritis, osteomyelitis and badly infected wounds, pneumonia (in aged particularly), pneumococcal mastoiditis, meningitis and empyema. In staphylococcal infections (especially with pus formation) which usually do not readily yield to sulphonamides, penicillin should be used. In gonorrhœa a routine course of penicillin is preferable and it is also drug of choice in gonococcal arthritis, ophthalmia, endocarditis, pericarditis, epydidimitis, prostatitis and peritonitis. *Cl. œdematis* reacts better to penicillin.

It has also been shown that sulphapyridine enhances the action of penicillin. Combination of penicillin with small amounts of sodium sulphathiazole results in considerably increased action of penicillin. The activity of penicillin is not interfered with nor enhanced by the presence of other antibiotics especially gramicidin. There is, however, considerable additive effect between penicillin and streptothricin.

Even sensitive bacteria become resistant to penicillin if they are grown in a culture media containing proportion of penicillin in insufficient quantities to prevent vigorous growth. In case of sulphonamides resistance can be readily produced both in vitro and in vivo if an infection is treated with insufficient doses. It has been shown that penicillin resistance only occurs very rarely in vivo.

Briefly it may be stated that the introduction of penicillin has resulted in decline in use of sulphonamides. The advantages of penicillin are that it acts in much higher dilutions in the body, its action is not hindered by the presence of pus and tissue lysate products, its margin of safety between the therapeutic and toxic doses is much greater and it places no burden on the already overtaxed vitality of the patient. Sulphonamides, however, are useful drugs and in some infections are more effective than penicillin. Their administration presents no difficulties and with some of the newer derivatives toxic effects have been considerably eliminated.

According to Medical Research Council War Memorandum No. 10 (1945), the following indicate the occasions on which sulphonamides should be given and those on which penicillin should be used when available:—

Staphylococcal infections.—Owing to their poor response to sulphonamide treatment, severe staphylococcal infections provide one of the strongest indications for treatment with penicillin. Acute osteomyelitis, cavernous sinus thrombosis, severe carbuncle, meningitis, septicæmia, endocarditis and pneumonia, due to staphylococci from whatever focus, call for penicillin treatment unequivocally and immediately. Sulphonamides should be tried in these conditions only if penicillin is not available.

Haemolytic streptococcal infections.—The vast majority of acute infections due to *Strept. pyogenes* respond well to treatment with sulphonamides; under present conditions of supply, penicillin should not be used unless adequate treatment with these drugs has failed. Dolphin and Cruickshank have recently reported encouraging results with penicillin in cases of acute bacterial endocarditis due to hæmolytic streptococcus groups A and B which do not respond to sulphonamides (Brit. med. J., 1945, i, 897).

Other streptococcal infections.—Microaerophilic streptococci, such as occur in osteomyelitis (particularly of the facial bones) and in suppurative conditions of the lung, are sensitive to penicillin, and these infections, therefore, justify its use; so does "spreading subcutaneous gangrene", resulting from a symbiosis of microaerophilic streptococci and staphylococci. Most strains of *Streptococcus viridans* are sensitive to penicillin, and in subacute bacterial endocarditis due to this organism a prolonged course of penicillin treatment is strongly indicated; the proportion of successes reported hitherto far exceeds that obtained with sulphonamides. *Streptococcus faecalis* is highly resistant to penicillin.

Pneumococcal infections.—In general, pneumococcal infections respond well to sulphonamides, and penicillin should not at present be used unless there is good reason for believing the organisms to be sulphonamide-resistant. Penicillin is, nevertheless, highly effective in pneumonia, and may be used more when supplies permit. In sulphonamide-resistant pneumococcal infections, particularly meningitis penicillin is strongly indicated.

Gas gangrene.—All three of the principal toxigenic *Clostridia* concerned in gas gangrene are more or less equally sensitive to penicillin, whereas their sulphonamide sensitivity varies greatly, *Cl. œdematiens* being notably resistant. True gas gangrene is, therefore, considered an unequivocal indication for penicillin treatment, though its value in this disease is not yet proven.

Meningococcal meningitis responds so well to sulphonamides that penicillin is rarely called for, though it is fully effective. In this, as in other forms of meningitis, penicillin if used, should be given intrathecally whenever possible; a short course of intramuscular injections should be given in addition, especially in bacteræmic cases.

Gonococcal infections.—A comparatively small total amount of penicillin, administered in a few intramuscular injections during a single day, will cure over 90 per cent of cases of uncomplicated gonorrhœa, whether acute or chronic and whether sulphonamide-resistant or not, and the remainder may be curable by further treatment with penicillin. Until recently the general policy has been to treat all cases of gonorrhœa in the first place with sulphonamides, and to give penicillin only for sulphonamide-resistant infections; nevertheless, the efficacy and simplicity of penicillin treatment is such that it is rapidly supplanting the sulphonamides for the treatment of this disease.

Syphilis.—Encouraging results with penicillin have been reported in the treatment of syphilis. The sulphonamides have no effect upon this disease.

Actinomycosis.—Strains of *Actinomyces* infecting man vary in their sensitivity to penicillin. The results of treating this infection have, nevertheless, been generally more strikingly successful than those obtained with sulphonamides.

Miscellaneous conditions.—Judging by the sensitivity *in vitro* of the causative organisms, *anthrax* and *diphtheria* should be influenced by penicillin treatment; little clinical evidence is yet available, but encouraging results have been reported in cutaneous anthrax (Murphy, La Boccetta & Lockwood: *J. Amer. med. Ass.,* 1944, 126, 948,). The response of sulphonamides to anthrax is uncertain. Diphtheria does not respond well to sulphonamide in any event, it should be treated mainly with antitoxin. Infection by *Viccent's organisms* is amenable to penicillin; the value of sulphonamides in this condition is doubtful, although good results have been claimed. There is experimental and some clinical evidence of an effect of penicillin in relapsing fever, Weil's disease, rat-bite fever due either to *Spirillum minus* or *Streptobacilus moniliformis,* and ornithosis (psittacosis). These conditions do not respond to sulphonamides.

Local applications.—What has been said hitherto about penicillin has referred mainly to systemic treatment, *i.e.,* the maintenance by intramuscular or intravenous injection of a concentration of the drug in the blood which is adequate for therapeutic effect. Penicillin has also a very wide sphere of usefulness as a local application. Its superiority to the sulphonamides for many purposes is due to three causes:—

(1) Its efficacy against even larger numbers of bacteria.

(2) The fact that nothing in inflamatory exudates antagonises it, as breakdown products in pus antagonise the action of most sulphonamides.

(3) Its almost complete freedom from tissue toxicity. The indications for local treatment with penicillin cannot be detailed here: the methods include injection into the subarachnoid space and into any of the serous sacs and joints; instillation into infected areas through tubes; the application of the drug in suitable media to wounds, burns, certain skin infections, certain infections of the eye, and susceptible infections of the mouth and throat. Wounds infected by haemolytic streptococci (unless they have been shown to be sulphonamide-resistant) should be treated by sulphonamides. Sulphonamides and penicillin are fully compatible, and the combined application of the two is often convenient and advantageous.

Conditions insusceptible to penicillin.—Nearly all gram-negative bacilli are resistant to penicillin in greater or less degree (among minor exceptions are *F. fusiformis* and the Morax-Axenfeld bacillus); these penicillin-resistant organisms include the enteric, food-poisoning and dysentery groups, some but not all strains of *Haemophilus,* the genera *Pasteurella* and *Brucella,* and several species commonly found in wounds (*Proteus vulgaris Ps. pyocyanea,* etc.).

Myco. tuberculosis is completely resistant to penicillin, and there is no evidence of therapeutic effect in any virus disease (with the possible exception of ornithosis), or in malaria or any other protozoal infection. Rheumatic fever is unaffected, as are all forms of chronic arthritis (so far as information goes) except gonococcal. Among other conditions for which penicillin, like the sulphonamides, has been tried without avail are cancer, leukaemia and lymphadenoma.

STREPTOMYCIN, NEOMYCIN, AUREOMYCIN, CHLOROMYCETIN AND OTHER ANTIBIOTICS

Antibiotics are substances elaborated by micro-organisms which act as antibacterial or antifungal agents. It is possible that these substances are formed as a defence mechanism. Pyocyanase was isolated from *Ps. pyocyanea* as early as 1889 and was suggested in the treatment of diphtheria and certain other infections. Pyocyanin was isolated from *Ps. aerogenosa.* Actinomycetin was isolated from strains of Actinomyces in 1924 and was found to be effective against certain gram-positive organisms. Citrinin was isolated from *P. citrinum,* Gliotoxin from *Tricradrine lignorum,* Fumigacin from *Aspergillus fumigatus* and clavecin from *Aspergillus clavatus.* Other similar substances named aspergillic acid, gignatic acid and flavicidin were discovered from *Aspergillus flavus* in 1943. Flavicidin was described in detail in 1944 and is similar to penicillin in its biologic characters. More recently a number of other useful antibiotic agents have been elaborated, prominent amongst them being aureomycin

derived from *Streptomyces aureofaciens,* neomycin isolated from an actinomycotic mould and chloromycetin which shows promise in the treatment of typhoid fever. All these substances show selective actions against certain micro-organisms. Thus far a dozen antibiotics have been described between 1939-1946.

Four main groups of antibiotic substances are available; (i) antibiotic agents of bacterial origin (spore and non-spore formers); (ii) antibiotic agents derived from moulds and fungii and (iii) antibiotic agents derived from Actinomycetes. (iv) Antibiotics of non-microbial origins.

1. Streptomycin

Waksman (1944) announced the discovery of Streptomycin, obtained from aerobic cultures of a ray fungus *Streptomyces griseus (Actinomyces griseus)* which he had isolated from the soil about 25 years earlier. Streptomycin resembles penicillin in its general properties but shows considerably greater activity against gram-negative and acid-fast bacilli.

Streptomycin is not produced by all strains of *S. griseus* and a number of substrains have now been obtained which are more effective producers of Streptomycin than the original culture. Meat extract or corn steep liquor are suitable media and the yield is increased with the addition of glucose to the medium. Isolation of the drug from cultures is effected by absorption on activated charcoal from which the drug is removed by acidified alcohol. This solution is neutralized with sodium hydroxide and ether and after further evaporation under reduced pressure, a highly concentrated aqueous solution of the drug is obtained. Streptomycin behaves chemically as an organic base in contrast to penicillin which is an acidic substance. The hydrochloride and sulphate are the salts commonly used. Pure base and pure crystalline salts have been prepared.

The original unit of Streptomycin was defined as the quantity of dry substance capable of inhibiting the growth of a given strain of *E. coli* in 1.0 ccm of nutrient broth or agar and was called the S unit, but the official unit now adopted is the activity contained in 0.001 mgm (1 microgram) of pure streptomycin base (which is equivalent to one original S unit). The potency of crystalline, streptomycin base is 1,000 micrograms per mgm and streptomycin issued for parenteral use should contain the equivalent of not less than 300 micrograms of the base per mgm of the dry powder, the moisture content of which should not exceed 3 per cent. Streptomycin hydrochloride or sulphate is now issued in containers containing the equivalent of 1.0 gm (1,000,000 units) of streptomycin base.

(1) Chemistry

Streptomycin is not a single chemical entity but a mixture of at least two chemically related substances: (i) One possessing the structure of an O-glycoside of the disaccharide streptobiosamine (N-methyl-a-glucosaminidostreptose) with streptidine (1, 3-diguanido—2, 4, 5, 6—tetrahydroxycyclohexane). (ii) The other is the pure streptomycin 'B' which contains an additional mannose residue and may be called mannosidostreptomycin. Thus chemical structure of streptomycin has been identified as N-methyl-l-glucosamide-streptosidostreptidine. Streptomycin compounds such as streptomycin B, and dihydrostreptomycin and streptomycin calcium chloride complex have recently been produced, and colorimetric and fluorometric methods of assay have been introduced.

Stability.

Streptomycin is freely soluble in water and in dilute acid solutions, but is insoluble in ether or chloroform. It is thermostable, the powder being stable at room temperatures for 6 to 9 months provided the moisture content is below 3 per cent. Solutions are very much less stable and freshly prepared solutions should be used for parenteral administration. It is more stable at acid pH level and may consequently survive passage through the stomach. It has been shown that though streptomycin is not inactivated by ferments similar to inactivation of penicillin by penicillinase, it can be inactivated by certain organic compounds such as cystine, S-aminoethanethiol and hydroxylamine. It is possible therefore to use cystine to distinguish between the two antibiotics and eliminate streptomycin from certain body fluids.

Activity.

In vitro, streptomycin shows considerable antibacterial activity against both gram-positive and gram-negative organisms. In the gram-positive group of organisms it is generally less active than penicillin, but unlike penicillin it shows considerable activity against the tubercle bacillus. In vitro, streptomycin possesses considerable antibacterial activity against such pathogenic gram-negative bacilli such as *Esch. coli, Aerobacter aerogenes, Eber. typhosa, Shig. dysenteriae, Pr. vulgaris* as well as organisms such as *H. influenzae, Kleb. pneumoniae, Ps. aeroginosa, Br. abortus* and *melitensis, H. pertussis, Past. pestis* and *tularensis* and *v. cholerae.* As is the case with penicillin, the activity of streptomycin is not considerably affected by the numbers of bacteria present. Blood, serum, pus or tissue breakdown products may however increase the tolerance of organisms to the drug by as much as four to eight times.

In vivo experiments, Feldman and Hinshaw (1944) and later Smith and McCloskey demonstrated the high efficacy of streptomycin in checking and retarding the normal course of experimental tuberculosis in guineapigs. Streptomycin was also shown to be very greatly superior to promin and was observed to possess a therapeutic index about ten times better than promin. Experimental evidence also demonstrated the value of streptomycin in the treatment. The effectiveness of streptomycin in other experimental infections has also been demonstrated. Prominent amongst these are *Dipl. pneumoniae, Past. pneumoniae, Past. pestis, Past. tularensis, Br. abortus, Kleb. pneumoniae, Borrelia novyi, Lept. icterohaemorrhagiae,* and *H. pertussis* and *influenzae.* It also exerts considerable protective effect against spirochaetal infections, though considerably less than penicillin.

(2) Absorption, excretion and distribution

Absorption.

Highest concentrations in the blood are obtained immediately following intravenous administration, about an hour after intramuscular administration and still later after subcutaneous injection. After oral administration detectable amounts do not appear in the blood stream, although high concentrations are found in the fæces. Streptomycin is also absorbed after inhalation in the form of spray and aerosols.

Excretion.

Streptomycin is excreted mainly by the kidneys, but more slowly than penicillin. This excretion has been shown to occur mainly by glomerular filtration and is therefore not delayed by drugs such as carinamide which delay tubular excretion of penicillin. It is also excreted in small amounts in the bile, fæces and sputum.

Streptomycin does not diffuse through the tissues as readily as penicillin, and low levels are found in the liver and spleen and none in the brain. In the blood its concentration is much higher in the plasma than in the blood corpuscles.

(3) Modes of administration

The high solubility and low toxicity of streptomycin enable it to be administered parenterally in much the same way as penicillin. Thus intravenous administration of 0.5 gm results in immediate high serum levels which fall progressively in 2 to 6 hours. Streptomycin is not excreted so rapidly as is penicillin. After systemic administration, the drug is readily absorbed and reaches the blood. It is excreted in the urine and is not detected in the fæces. After oral administration, very little is found in the blood stream or in the urine but large quantities are found in the fæces. Small quantities diffuse into the cerebrospinal fluid after systemic administration of a large dose. Streptomycin passes readily through the placenta and reaches the fœtal circulation after administration to pregnant women.

Streptomycin may be administered by mouth when it is intended that its effect should take place on organisms present in the gut; 0.5 gm to several gms may be given per day by this route.

(1) *Subcutaneous route.* 0.5 gm in 1 to 5 ccm of normal saline or distilled water may be given every 4 hours or at longer intervals. There is less local irritation than with penicillin, and also less pain than when the intramuscular route is used.

(2) *Intramuscular route.* In general, the intramuscular route is the most satisfactory one and the same technique as for penicillin therapy is advised. Due to the slower rate of excretion, however, it is not necessary to administer the drug more frequently than every 4 hours. Recent studies indicate that the frequency of administration can be varied greatly, and that when the total daily dose is at least 1 gm, results are often comparable whether the drug is given at four, six, eight, twelve or twenty-four hour intervals. In order to maintain a satisfactory bacteriostatic concentration in the blood, it is probably necessary to give the drug every 6 hours. Garrod (1948) suggests that in relatively large doses, streptomycin is actually bactericidal and that infrequent administration is effective due to the destruction with each dose of a proportion of the bacteria population. Recent studies in the therapy of tuberculosis indicate that the entire daily dose of streptomycin can be given in one injection without adverse effect on the therapeutic response.

(3) *Intravenous route.* Either by continuous drip method or by intermittent injection, as much as 4 gm have been given in a day. Local irritation occured at the site but was not serious. But concentrated solution should not be administered by this route because of the occasional presence of physiologically potent impurities which may produce serious results. This route possesses no advantage over the subcutaneous or intramuscular routes and is not commonly used.

(4) *Intrathecal administration.* 0.1 gm may be given dissolved in 5 to 10 ccm of normal saline or distilled water every 24 to 48 hours.

(5) *By spray.* Streptomycin can be given satisfactorily by nebulization in the same way as penicillin 0.05 gm being introduced into the nebulizer and inhaled. 0.5 gm daily may be given by this method for weeks.

(6) *Local administration.* Streptomycin may be used locally and may be injected directly in the empyema and abscess cavities. Concentrations of the drug of 10,000-100,000 units (10-100 mg.) per c.c. in isotonic solution of sodium chloride have been used.

(7) *Topical application.* In the treatment of empyema or meningitis, a concentration of 10,000-100,000 units (10-100 mg.) is directly introduced into the pleural cavity or subarachnoid space.

Toxicity.

(4) Toxic effects

Toxic effects are frequently encountered in streptomycin therapy especially with larger doses and this constitutes one of the chief drawbacks of the drug. Minor toxic effects are due to allergic reactions either to the streptomycin or to associated impurities. Acute histamine-like reactions such as flushing, nausea, vomiting, headache and an abrupt fall of blood pressure, may follow intravenous or intramuscular administrations in previously sensitized persons. Local irritation at the site of injection is common and appears to be due chiefly to impurities and is not influenced by simultaneous use of procaine hydrochloride. Irritation of the renal tract is common, but serious damage leading to anuria occurs only with very high dosage especially where previous kidney damage already exists.

Various manifestations of sensitisation reactions characterised by fever and skin erruption of erythematous, urticarial, maculopapular or even hœmorrhagic type, (but usually a pruritic erythematous maculopapular type), at first localised are encountered; these are relieved by benadryl. Treatment need not be stopped unless it develops into an ex-foliative dermatitis. Most of the rashes appear from the 3rd to the 10th day of treatment. They usually last for only one day but in some cases persist for 7-9 days. Contact dermatitis affects hands as also face and conjunctiva; sometimes this is so severe as to induce persons affected to wear gloves.

The most serious toxic effect encountered with streptomycin when large doses are given for prolonged periods is complete and permanent loss of vestibular function. With smaller doses only partial or temporary disfunction may result. Deafness is rare, and is met with only in patients treated for tuberculous meningitis by intrathecal injections. Paresthesias, particularly about the facial region, are common but are usually mild and transitory. Psychoses may occasionally be met with.

It has been noted that even moderate but continuously sustained concentrations of the drug are more likely to cause toxic manifestations than high peak levels with low levels in between as when the total daily dose is given in one or two injections. The newer compound, dihydrostreptomycin appears to be less liable to produce neurotoxic symptoms than ordinary streptomycin.

Assay.

Standardisation. (i) Microbiological assay—It is usually carried out by the agar diffusion method or the so-called cup method, in which either a standard strain of *Bacillus subtilis* or *staphylococcus aureus* is used as the test organism. The activity of an unknown preparation is determined by comparing the zones of inhibition produced with that of the standard.

(ii) Test for toxicity in mice—For crude preparation of Streptomycin toxicity was found as follows:—LD_0=35 mg. and LD_{100}=135 mg. per 20 gm. mouse by intraperitoneal injection. To protect 18 to 20 gm. mouse infected with *U. Schottmulleri* 6.5 mg of the crude material is usually required. For pure streptomycin subcutaneous toxicity in mice is about 1,500 mg/kg.

Precautions in using streptomycin. One of the most important points that should be considered in using maximum doses is the so-called 'stimulating effect

of steptomycin' on the growth of bacteria. Streptomycin at certain concentration levels when injected intraperitoneally in mice infected with *E. typhosus* increases rather than decreases the fatality rate.

(5) Therapeutic uses of streptomycin

(1) *Extrapulmonary tuberculosis.* In generalized miliary tuberculosis where there is no involvement of the meninges, streptomycin produces remarkable results, and though complete and permanent cure is not attained, striking remissions usually result, which may last for as long as two years or more. A total daily dose of 1 to 2 gm divided into two equal doses should be given intramuscularly morning and evening, and should be continued for two to six months according to the response.

Meningitis.

In tuberculous meningitis, dramatic remissions are often effected by streptomycin, though the ultimate fatal outcome is rarely prevented. The remissions may persist for many months. Streptomycin should be administered by the intrathecal route in addition to intramuscular injections in the dosage given above. 0.1 gm dissolved in 5 to 10 ccm of normal saline or distilled water may be given every 24 to 48 hours. Intensive parenteral and intrathecal streptomycin therapy is advised, a child receiving 200,000 units intrathecally once a day and 100,000 units intramuscularly every 3 hours. 27 days after treatment started intrathecal administration is discontinued and the daily intramuscular dose is increased by 100,000 units *i.e.* total daily dose given is 200,000 units.

Peritonitis.

Tuberculous peritonitis and enteritis respond very favourably to streptomycin, symptomatic improvement being usually manifest within a couple of weeks and complete healing may occur.

Tuberculous cutaneous sinuses heal quite rapidly and consistently and recurrences are rare. Enlargement of lymphatic glands if present also retrogresses and the infection becomes quiescent. Anal fistulæ respond satisfactorily if not of long duration. Tuberculous pleural fistulæ often prove refractory to treatment though it has been claimed that healing occurs in more than fifty per cent of cases.

Genito-urinary tuberculosis.

In the treatment of genito-urinary tuberculosis although pyelographic studies seldom show any improvement in the renal lesions, prolonged symptomatic improvement is usually effected in the great majority of cases. Bladder lesions show marked improvement and associated sinuses heal. The urine also becomes free of tubercle bacilli in a high per cent of patients. In tuberculosis of the kidney the value of streptomycin therapy is chiefly as an adjunct to operation. Streptomycin alone may arrest the disease process for a few months but permanent cure can only be expected by excision of the diseased organ.

In tuberculosis of the bones and joints streptomycin has proved useful, either alone or in combination with surgical measures.

Otitis media.

Tuberculous otitis media responds extremely well to streptomycin and has proved a boon in this hitherto very refractive disease. Combined parenteral and topical treatment is suggested in severe cases of tuberculous laryngitis and ulcerating tuberculous lesions of the mucous membrane of the oropharynx. In progressive ulcerating tuberculous lesions of the tracheo-bronchial tree, however, streptomycin will not benefit fibrous strictures of the tracheo-bronchial passages.

Empyema.

Streptomycin has not proved to be of any value in tuberculous empyema, due presumably to the highly acidic nature of the empyema fluid but the drug is considered to be useful in tuberculous pleural effusion. Treatment should not however be prolonged beyond two to three weeks.

(2) *Pulmonary tuberculosis.* In general it may be stated that while streptomycin is of unquestionable value in the treatment of selected cases of pulmonary tuberculosis, considerable difference of opinion at present exists about the type of lesions where drug should be employed as a routine. In practice, lesions which are accessible to the blood stream and uncomplicated by irreversible anatomical changes are most benefitted; such lesions are mainly exudative, early and usually due to bronchogenic or hæmatogenic dissemination. Because of the regularity with which streptomycin produces resistant strains of the tubercle bacillus its use is not advisable in such cases as would have a good prognosis without chemotherapy. Chronic fibroid and fibrocaseous tuberculous lesions are also not suitable for streptomycin therapy. Ulcerating lesions of the bronchi and trachea are very favourably influenced as also is tuberculous laryngitis and ulcerating lesions of the mouth and pharynx.

Perhaps the greatest value of streptomycin in pulmonary tuberculosis is that it makes surgical treatment feasible in such cases where this would otherwise not be possible. Thus in fibrocaseous and fibrocavernous disease, streptomycin by causing resolution of exudative lesions especially in the opposite lung, reducing toxaemia and improving the general condition of the patient, may make such radical surgical measures as pneumonectomy and thoracoplasty possible and reasonably safe. Pneumothorax can often be established more expeditiously and safely in acute pneumonic tuberculosis after a course of streptomycin.

Administration, dosage and toxic effects. In earlier days, streptomycin was administered to tubercular patients in doses ranging from 1 to 3 gms a day for two to six months and toxic reactions especially damage to the vestibular apparatus were frequently encountered. Recent investigations, however, have demonstrated that streptomycin in daily dosages of 0.5 to 1 gm apparently yields as good results as were obtained with the higher doses employed previously. It has also been shown that when the total daily dose is at least 1 gm, the therapeutic response is as good whether the drug is given at intervals of 4, 6, 8, 12 or 24 hours. Thus it appears likely that the total daily dose can be given in one single daily intramuscular injection. With this reduced dosage and increased interval of administration, the incidence of toxic reactions is also greatly decreased.

Bacterial resistance in tuberculosis. One of the chief disadvantages of streptomycin therapy in tuberculosis is the ease and rapidity with which highly resistant strains of *Myc. tuberculosis* develop. In a recent study Pyle (1947) found that in approximately 75 per cent of cases investigated, the bacterial population became predominantly resistant after six to sixteen weeks of treatment, and bacilli resistant to such high concentrations as 3,000 micrograms of streptomycin per ccm of medium have been recovered from patients. It is because of this development of bacterial resistance that the concensus of opinion at present is that in most cases the administration of streptomycin should be limited to five or six weeks or even less. In relapses, treatment can be resumed if the organisms are still sensitive to streptomycin in vitro.

Contra-indication of streptomycin therapy in tuberculosis:—

(i) Chronic fibroid or fibrocaseous pulmonary tuberculosis.

(ii) Acute destructive and apparently terminal type of tuberculosis.

(iii) Minimal or early moderately advanced pulmonary tuberculosis with favourable prognosis.

(iv) Chronic empyema of tuberculous origin.

(v) Streptomycin treatment should be avoided when other treatments are available, because to produce a drug resistant strain of tubercle bacilli by such treatment may possibly make any other form of treatment ineffective, should a more serious type of tuberculosis subsequently develop.

(3) *Brucellosis.* Spink et al (1948) consider a combination of sulphadiazine and streptomycin an effective form of therapy for human brucellosis. They advise a dosage of streptomycin 2 gm per day (0.5 gm every 6 hours intramuscularly) for 7 to 12 days, together with sulphadiazine 4 gm initially and 1 gm every 4 hours thereafter, for 2 to 3 weeks. Hielmán (1949) in a study on mice infected with brucella organisms showed that streptomycin or dihydrostreptomycin, in combination with aureomycin was most effective. Herrel and Barber (1949) obtained very good results in a small series, using dihydrostreptomycin 2 gm a day in divided doses intramuscularly, combined with aureomycin 3 gm a day by mouth in divided doses every 6 hours. All patients promptly recovered without recurrence.

(4) *Tularaemia.* Streptomycin yields very good results and for adults 2 gm daily in divided doses is continued for 5 to 8 days. For children and adults below 50 kg in weight the total daily dosage should be 0.04 gm per kilo body weight. The drug should be stopped if deafness, vertigo, etc. appear.

(5) *Hæmophilus influenzæ pneumonia.* A combination of streptomycin and sulphadiazine gives good results. The usual dosage of sulphadiazine is combined with 2 to 3 gm per day divided into six 4-hourly doses intramuscularly.

(6) *Kliebsiella* (Friedlander) *pneumonia.* Sulphadiazine is given together with streptomycin 0.5 gm every 4 hours (3 gm per day) till the sensitivity of the infecting organism can be determined, after which the dose may be increased if necessary, so as to maintain a blood concentration at 4 to 8 times this level. The treatment is continued for three days after the temperature falls to normal. Streptomycin is employed in the same way for lung abscess due to Friedlander and colon bacillus organisms.

(7) *Granuloma Inguinale.* Streptomycin has proved to be the most valuable drug so far for this condition. A dosage of 4 gm per day is given in divided doses every 4 hours for 5 days, but with this high dosage a careful watch must be kept for toxic effects. Recurrences may respond to a second course of streptomycin.

(8) *Subacute bacterial endocarditis.* Where the causative organisms are resistant to penicillin, *e.g.,* infections with *Esch. coli, Aerobacter aerogenes, Hæmophilus influenzae, Kliebsiella pneumonia, Brucella, Past. pestis, Pseudomonas aeruginosa, etc.,* streptomycin is given in doses of 2 to 5 gm daily in divided doses for six to eight weeks.

(9) *Acute appendicitis.* For perforated appendix, streptomycin 2 gm a day in divided doses is given for 3 to 5 days postoperatively in combination with penicillin 400,000 to 800,000 units a day. In the conservative treatment of appendix abscess, give streptomycin 1.5 to 2 gm a day in addition to penicillin

400,000 units per day. The patient must be carefully watched for signs of spread of infection or rupture, when of course recourse must be had to immediate operative interference.

(10) *Bronchiectasis.* Antibiotics are of value in controlling associated acute superimposed respiratory infection and in preparing patients for pulmonary resection or thoracoplasty. Penicillin and streptomycin either alone or in combination may be used. Streptomycin 0.5 gm dissolved in 20 ccm of normal saline should be nebulized in 3 or 4 sessions every 24 hours. In addition 0.5 gm of streptomycin is given daily by intramuscular injection.

(11) *Chronic ulcerative colitis.* In the acute and subacute toxic phases, streptomycin 0.5 gm 4 times a day should be started immediately together with sulphadiazine 1 gm orally, 4 times a day. Both the drugs should be continued for two weeks.

(12) *Agranulocytosis.* The causative drug should be stopped immediately and penicillin 40,000 units given every 3 hours till the neutrophils return to the circulation in normal numbers. If the infecting organisms are sensitive to it, streptomycin, 0.25 to 0.5 gm may be given in addition every three hours.

(13) *Plague.* A large number of recent reports have confirmed the value of streptomycin in the treatment of bubonic and septicaemic plague. Streptomycin 2 to 3 gm daily in divided doses every 4 to 6 hours is administered together with sulphathiazole or sulphadiazine orally. Both drugs should be continued for at least three days after the fall of temperature to normal. Suppurating buboes are dealt with surgically as necessary.

Septic abortion.

(14) *Streptomycin in miscellaneous surgical conditions.* In the treatment of septic abortion, if response to penicillin and sulphonamide therapy has been unsatisfactory or if culture shows susceptible gram-negative organisms, streptomycin 1 gm daily may be given in 3 or 4 divided doses.

Cholecystitis.

In acute cholecystitis with perforation or impending perforation, in preparing the patient for operation, streptomycin 0.5 gm intramuscularly every 4 hours may be given instead of penicillin or if the organisms cultured from the peritoneal cavity are penicillin resistant.

In the initial conservative treatment of acute pancreatitis, streptomycin 0.5 gm every 4 hours may be given instead of penicillin.

In acute mastoiditis, if there is failure to improve with penicillin or sulphadiazine and the infecting organism is streptomycin sensitive, give streptomycin 0.3 gm every 3 hours day and night for adults, and 0.02 gm per kilo body weight daily for children below 40 kg. Development of deafness or vertigo are indications for stopping the drug.

For chronic otitis media, wicks moistened with streptomycin, 2,500 units per ccm, are effective in infections due to *B. pyocyaneus.* Treatment should not be continued beyond ten days due to the risk of development of skin sensitivity.

In the treatment of acute regional ileitis, it has been reported that approximately 25 per cent of cases resolve with the combined oral and parenteral use of streptomycin. The drug should be given in doses of 1 gm a day by mouth combined with 0.5 gm 4 times a day by intramuscular injections.

In the post-operative treatment of cerebral abscess, if the organism is not sensitive to penicillin, streptomycin should be given in doses of 0.25 gm every 3 hours for two weeks.

In preparing patients for the surgical treatment of intestinal tumours, streptomycin should be given orally, 0.5 gm every 6 hours for 48 hours before the operation till the temperature has been normal for 24 hours.

In pyogenic liver abscess, streptomycin 0.25 gm every 3 hours may be given in addition to penicillin 100,000 units every 3 hours. If with this regimen the fever does not subside in 2 or 3 days, immediate operative interference is called for.

2. NEOMYCIN

Stability.

Streptomycin has two serious disadvantages in the therapy of tuberculosis: its toxic effects on the central nervous system particularly in the 8th cranial nerve, and the ease with which it produces resistant strains of the tubercle bacillus. After examination of thousands of cultures of actinomycetes from the soil belonging to the genus *Streptomycin fradiae* (*Actinomyces fradii*) Neomycin is isolated by Selman A. waksman from cultures by methods of absorption and elution similar to those employed for streptomycin. It is a basic water soluble compound and most active at an alkaline solutions which is stable to heat and is active against a wide range of gram-negative and gram-positive organisms, especially mycobacteria but not against fungi. It is insoluble in organic solvents. In vitro tests show it to be equally active and in some cases even more active against streptomycin sensitive and streptomycin resistant strains of tubercle bacilli. Studies thus far have demonstrated its low toxicity and lack of tendency to produce resistant strains.

The following is a list of organisms susceptible to the action of neomycin experimented upon so far:—

Activity.

Escherichi coli, Bacillus subtilis, B. mycoides, Staphylococcus aureus, Mycobacterium 607, M. ranae, M. avium, M. phley, B. cereus, S. lutea, Ps. aeruginosa, Pr. vulgaris, Bodenheimer's organism, Serratia marcescens, Mycobacterium 607Rs, Trichophyton mentagrophytes, Candida albicans.

Neomycin preparations are found to possess several desirable properties:— (a) similar activity against both streptomycin sensitive and streptomycin resistant bacteria; (b) considerable activity (in some cases greater activity than streptomycin) against various forms of *M. tuberculosis* and other mycobacteria; (c) limited toxicity to animals or none; (d) activity against various bacteria *in vivo,* including gram-positive organisms and against both streptomycin sensitive and streptomycin resistant organisms; (e) lack of resistance against neomycin among the organisms sensitive to it, or only limited development of such resistance.

Since neomycin has not yet been obtained in crystalline form, very little can be said of its chemical nature. Preliminary results, however, point to its being distinctly different chemically from streptothricin and form streptomycin.

3. AUREOMYCIN

Aureomycin is an antibiotic derived from a strain of *Streptomyces aureofaciens.* It was discovered by B. M. Duggar while working at the Lederle Laboratories Division of the American Cyanamid Company. The antibiotic is faintly golden yellow. Aureomycin has an unusual degree of potency and range of activity, acting against many Gram-positive and negative organisms. However, the most outstanding feature of aureomycin is its activity against many Rickettsiae, and certain viruses.

(1) *Chemistry*:—Aureomycin is a weakly basic compound which contains both nitrogen and nonionic chlorine. Aureomycin when treated with alcoholic ferric chloride gives a greenish brown colour by reflected light and reddish colour by transmitted light. The crystalline free base has the following properties: m.p., 168-169°C (uncorr.). Solubility in water, 0.5-0.6 mgm. per ml. at 250°C; very soluble in the cellosolves, dioxane, and carbitol. Very soluble in aqueous solution above pH 8.5. Somewhat less soluble in isotonic sodium chloride solution. These solutions are acid (pH 4.5). Aureomycin is soluble in acid and alkaline solution but is almost insoluble at pH 7. It is available as a yellow crystalline hydrochloride salt.

Stability. The activity of this antibiotic deteriorates rapidly in alkaline solutions at room temperature. At pH 2.5, the substance is stable but at pH 8.5, 25°C. it loses its activity. Aureomycin kept in dry powder form in sealed ampoules or in capsules retains its potency for at least seven months at room temperature. Solutions kept frozen at 20°C retain their activity for long periods.

Absorption. After oral administration, aureomycin is rapidly absorbed through gastro-intestinal tract. The oral route of administration is preferred because the drug is promptly absorbed and the results are uniformly consistent.

Blood concertration. A qualitative assay is difficult, but quite low levels like 0.3-2.5 mg. per c.c. have been detected in the blood serum. The plasma levels, after oral doses of upto 1 gm given six hourly, have usually been found to be about 2 micrograms per millilitre or less, but the methods of estimation however were not satisfactory. Harnod and co-workers (1949) found that in therapeutic dosage in dogs aureomycin readily passes the blood-brain barrier and substantial amounts remained in the blood stream (4.0 micro-grams per c.c.) and in the cerebrospinal fluid (0.8 micro-grams per c.c.) two hours after an intravenous injection. Schoenbach and co-workers (1949) found 1.2 to 2.4 micrograms per c.c. in the serum following an intramuscular injection of 40 mgm in patients who had been on therapy for 5 to 14 days. Wright and co-workers (1948) found that the highest blood levels occured two hours after oral administration of 300 mgm, 2 micrograms per c.c. of aureomycin.

Excretion. Aureomycin is very rapidly excreted in the urine and the excretion continues for 2-3 days after a single dose of 0.5-0.75 g. Levels upto 320 gm. per c.c. have been detected after oral administration. The greatest rate of excretion in the urine occurs between 4-8 hours after oral administration. The rate of excretion remained relatively constant for at least 6 hours, while after intravenous injection the excretion was rapid during the first two hours, slower between the second and fourth hours, and low between the fourth and sixth hours.

(2) *Toxicity*:—The acute toxicity of aureomycin was determined in mice, rats, rabbits and dogs. The oral toxicity was found to be low, the mortality in mice being only 5 per cent at a dosage of 2,500 mg per kilo. The LD_{50} on intravenous injection in mice is between 50 and 100 mgm. per kilogram of body weight. The LD_{50} on subcutaneous injection in mice is between 3,000 and 4,000 mgm. per kilogram of body weight. Rapid intravenous injection of 150 mgm. per kilogram of body weight killed a dog and produced haemoglobinuria. Repeated large doses (20-50 mgm. per kg. of body weight) of aureomycin were tolerated in rats and dogs, with moderate local reaction and some loss of weight associated with anorexia. Autopsy revealed no gross or microscopic abnormality of the viscera.

Toxic manifestations in human beings—Toxic effects were minimal and infrequent, except after intravenous administration. As a whole the drug is well tolerated and is relatively non-toxic for all practical purposes, when given in therapeutic doses. The commonest complain during large doses of aureomycin are looseness of the bowels with frequent and bulky stools, but true diarrhoea is uncommon. Nausea and occasionally vomitting occured after one or more doses in a few patients. Anæmia attributable to the drug did not occur, and no depression of the cells in the granulocytic series is observed. There is no evidence of renal irritation, fever or rashes during the period of aureomycin therapy. In a few cases of cystitis a disagreeable "drawing" or "squirming" sensation was noted in the pelvis which may be related to the high urinary acidity. No neurological abnormalities like nystagmus, vertigo, tinnitus or auditory disturbances have been observed. An intramuscular injection leads to local irritation and variable degrees of pain, from moderate to severe, and lasting from several minutes to several hours. Patients and normal individuals, ranging in age from 34 to 45 years, have been given from 15 to 30 mgm per kg of aureomycin daily, over a period of 5 to 20 days. Braley and Sanders (1948) stated that, while aureomycin is moderately irritating when injected intramuscularly, the addition of a small amount of procaine hydrochloride almost completely prevented discomfort. Topical administration of aureomycin in patients with staphylococcal ophthalmic infections, indicated that an ointment containing the hydrochloride was irritating to nearly all patients, although active against the infection, while an ointment prepared with the Borate was non-irritating but probably quickly lost its activity. *Toxic symptoms.*

(3) *Factors Influencing Aureomycin Activity*:—Aureomycin is effective only against vigorously multiplying organisms but not against fully grown or resting cultures. Aureomycin is much more effective in an acid than in an alkaline medium the reverse of streptomycin. Filtration of solutions of aureomycin in water, broth or urine through Seitz, Mandler or sintered glass filters, does not appreciably reduce the activity of aureomycin.

There is no significant tendency for the development of resistance to aureomycin in organisms either *in vitro* or *in vivo*. This is in sharp contrast to streptomycin. All organisms of the same species, isolated from the same patient, before during or after the treatment with aureomycin for varying periods upto one month or longer, were equally sensitive to this antibiotic. *Resistance.*

(4) *Action on Micro-organisms*:—Aureomycin possesses bacteriostatic and bactericidal activity against numerous gram-positive and gram-negative bacteria. Its activity deteriorates rapidly in alkaline solution. Human serum also decreases its activity. The following organisms are susceptible to aureomycin:—

(1) β-haemolytic streptococcic strains, Group A, D, F and G.
(2) *Diplococcus pneumoniæ,* Types, I, II and III.
(3) Staphylococci.
(4) Some strains of *B. coli aerogenes.*
(5) *Klebsiella pneumoniæ.*
(6) *H. influenzæ.*
(7) *Brucella suis and abortus.*

Pseudomonas aeruginosa and strains of Proteus are not inhibited even in high concentrations.

(5) *Indications for use*:—Aureomycin hydrochloride (Lederle) is a yellow crystalline antibiotic potent against many gram-negative and gram-positive organisms as well as highly specific against Rickettsial infections. It is also

effective against primary atypical pneumonia, an infection of unknown etiology. Aureomycin should be used against infections that have become resistant to penicillin, streptomycin, or sulfonamides and in patients who exhibit severe and uncontrollable sensitivity to these drugs. The following are the diseases acted upon successfully by aureomycin:—

(a) Rocky mountain spotted fever:—This disease is caused by *Rickettsia rickettsii*, the vector being the common tick. Aureomycin appears to be specific for this organism. Wong and Cox (1949) found that this drug possesses marked effectiveness against the rickettsiae of spotted fever, typhus fever, scrub typhus fever and Q fever in chick embryos. The drug is given orally initially in doses of 0.5 gm. every 6 hours followed by slightly higher doses for 4 days. Temperature responds in about 3 days, the only side effects during the administration of the drug is large loose motions found in very few of the patients.

(b) Q fever:—This is an acute infectious disease of rickettsial type caused by *C burnetii* and prevalent particularly on the west coast of the United States and Canada. Aureomycin is given in oral daily dosage varied from 2 to 5 grains. Cases running a febrile course for many days before treatment required a correspondingly longer period of treatment, whereas cases that had been, febrile for only a few days required a correspondingly brief period to restore the temperature to normal.

(c) Rickettsial pox:—This relatively new disease, an outbreak of which has recently occured in New York City, appears to be controlled by aureomycin.

(d) Exanthematous typhus:—An average daily dose of 200 mgm. per kg of body weight are given orally over a period of 36 hours (the total dose being 300 mgm per kg of body weight). The results are excellent. Within the first few hours of treatment headache and muscle pain usually disappear and in 36 hours temperature usully comes down to normal.

(e) Recrudescent epidemic typhus (Brill's disease):—The initial dose was 200 mgm orally every hour for 3 doses and thereafter every 2 hours day and night.

(f) Primary atypical (virus) pneumonia:—1.5 gm orally as initial dose is given followed by 1 gm every 6 hours and continued for several days after the temperature has become normal.

(g) Pyaemia and osteomyelitis.

(h) Pyoderma gangrenosum in chronic non-specific ulcerative colitis.

(i) Virus diseases like lymyhogranuloma venereum and psittacosis ("Parrot fever"):—Aureomycin appears to improve markedly acute ulcerative and inguinal forms of lymphogranuloma venereum. Chronic cases with advanced perirectal and similar changes require various adjuvant measures, including surgery. Aureomycin was stated to be the treatment of choice in all cases of lymphogranuloma venereum infections—together with surgery whereever mechanical conditions demand it. Wong and Cox (1949) have shown in experimental animal that aureomycin possesses marked therapeutic activity against the entire group of psittacosis—lymphogranuloma viruses.

(j) Bacterial infections (including many gram-positive and negative organisms, acute and chronic Brucellosis, due to *Brucella melitensis*, Tularaemia, sulphonamide, penicillin, streptomycin resistant organisms, Coli aerogenes group of infections of the urinary tract and peritoneum).

(k) Ocular infections (bacterial and viral).

(i) Bacterial infections:—Braley and Sander (1948) have recently reported at aureomycin borate is effective in 0.5 per cent concentration in staphylococcic, eumococcic and influenzal conjunctivitis. It is also effective in infections used by the diplobacillus of Morax-Axenfeld and Friedlander's bacillus.

(ii) Virus like infections:—Several eye infections that are thought to be ral like in nature respond to aureomycin including conjunctivitis, follicular njunctivitis and herpes simplex corneae (dendritic keratitis).

Ophthalmic infection.

Ophthalmic infection may usually be controlled by the use locally of an phthalmic solution made by adding 5 c.c. of distilled water only to 20 mgm of ureomycin contained in a dropper bottle designed for this purpose by Lederle. f this solution be kept under refriegerator conditions, it will remain stable for pproximately two days. Combined local and oral therapy is preferred in iplobacillus (Morax-Axenfield) ulcers, Friedlander bacillus ulcers and in eneral where there is severe infection. One or two drops should be placed n affected eye or preferably in both eyes, every two hours or oftener, depending pon the severity of the infection. Aureomycin hydrochloride ophthalmic olutions should be made up fresh every two days.

(l) Gonorrhœa:—If the gonococcus become resistant to penicillin, ureomycin may be used. In order to equal the effect produced by a single njection of 300,000 units of repository penicillin, the total dose of aureomycin hould be greater than 1.5 gm and be given over a period of 2 days. Chen and co-workers have called the attention of the medical profession to the fact that ureomycin is the first chemotherapeutic agent to have been reported effective gainst all 5 venereal diseases, namely, lymphogranuloma venereum, gonorrhœa, granuloma inguinale, chancroid and syphilis.

(m) Meningococcus:—Collins and co-workers have reported a case of meningococcemia in which aureomycin was begun 12 hours after the onset of symptoms. The dosage employed was 500 mg for 4 doses, 350 mgm for 4 doses and 250 mgm for 3 doses at 6 hour interval.

(n) Escherichia coli:—Fine, Seligman and Frank have stated that aureomycin is the most effective drug now available for E. coli peritonitis.

(o) Bacteroids Funduliformis septicaemia:—Sprout and co-workers have successfully treated cases of *Bacteroids Funduliformis* septicaemia with aureomycin.

(p) Infections caused by hæmophilus organisms:—*Hæmophilus pertusis*—At present aureomycin is the most promising agent for the control of this infection. 0.5 gm of aureomycin per kilo being given in devided doses over a period of 4 to 8 days.

Hæmophilus influenza:—It is equally effective against *Hæmophilus influenza.*

(q) Syphilis:—Relief of cutaneous syphilides and of complicating fusospirillary infections have been obtained but the serology seem to have been unaffected. Prompt healing of the primary and secondary lesions usually took place with a marked quantitative changes in the Kahn reactions.

Aureomycin has been found useful, against the following organisms, but its full therapeutic value has not yet been determined:—

(a) Salmonella organisms (the drug is effective in large doses).

(b) Gram-positive cocci other than those that are penicillin resistant.

Aureomycin is found ineffective in the following conditions:—

Proteus vulgaris and *Pseudomonas aeruginosa;* B-strain of influenza, canine distemper, rabies, Newcastle disease, Venezuelar equinine encephalomyelitis and M E F-1 strain of poliomyelitis.

(6) *Doses*:—For severe infections like Rickettsial infections and Q. fever, 50-100 mg per kg of body weight per day given orally in divided doses. 300-500 mg per kg per day have been given in typhoid fever and other salmonella infections without untoward results.

Moderate Infections.

For moderate infections like primary atypical pneumonia and most other infections, 25-50 mg per kg of body weight per day, orally, in divided doses. This dosage should be continued for 5-14 days as judged by the clinical response. Usually, it is given every four hours for the first 24 hours and then six hourly. If definite therapeutic effects occur, the dosage can be reduced to a lower range within a few days. Intramuscular injections should be reserved for cases that are moribund or for fulminating infection.

Aureomycin has definite antibacterial activity against the many micro-organisms, including coccic and bacillary forms and it may prove useful in the treatment of infections with organisms that are resistant to penicillin or to streptomycin. Aureomycin given orally, although has beneficial effect in coccic infections but the results in cases of gonorrhœa are inferior to those obtained with adequate doses of penicillin. The result in some of the patients with typhoid and salmonella infections are favourable. The observations of some of the eminent workers both in experimental animals and in human beings suggest that the major field of usefulness of aureomycin may be in the treatment of infections with rickettsias or with viruses of the psittacosis-lymphogranuloma venereum group. The only advantage is that resistant organisms do not develop during treatment of aureomycin.

4. CHLOROMYCETIN (CHLORAMPHENICOL).

(1) *Introductory*—The isolation of a new antibiotic substance, chloromycetin, from a soil actinomycete was reported by Ehrlich, Bartz et al in 1947. It is a pure crystalline substance possessing specific antibiotic activity and has been obtained from cultures of species *streptomyces venezuelae* as discovered by a group of workers from the Research Laboratories, Parke Davis & Co. It has also been prepared synthetically. Oral chloromycetin therapy is effective against enteric infections, notably typhoid fever and other salmonella infections, bacillary urinary infections and many rickettsial and viral diseases, including atypical (virus) pneumonia, psitacosis and lymphogranuloma venereum. The drug is available as 0.25 gm. kapseals and is administered orally.

Stability.

Chloromycetin is a neutral compound and is unique in that it contains both nitrogen and nonionic chlorine. It is further more characterised by being stable at room tmperature in aqueous solution over the pH range of 2-9 for more than 24 hours, and in distilled water is unaffected by boiling for 5 hours.

(2) *Antibacterial activity*—In vitro studies indicate that chloromycetin is seven to thirty six times as active as penicillin against a large number of pathogenic gram-negative organisms, though it was much less active against *Staph. aureus.* It was only about one tenth as active as Streptomycin against sensitive strains of *Myc. tuberculosis.* It showed great activity against *Borellia recurrentis* and a large number of rickettsial and viral organisms including those of atypical pneumonia, psittacosis and lymphogranuloma venereum.

In vivo experiments indicate the powerful antibacterial activity of chloromycetin against a wide range of infections, notable amongst which are the rickettsial infections, enteric infections notably typhoid fever and other salmonella infections, bacillary urinary tract infections, and the virus infections of primary atypical pneumonia, psittacosis and lymphogranuloma venereum. Experimental infection in mice with *Vibrio cholera* and *Haemophilus pertussis* respond to moderate doses of chloromycetin. The drug has proved ineffective against protozoal infections.

Absorption,

(3) *Administration, dosage and toxic effects*— 'Chloromycetin is well absorbed after oral administration and this is the route of choice that is usually employed. The drug can also be administered intravenously and this route may be employed in addition to the oral route in very acute cases.' The dosage employed varies with the type of infection and generally is of the order of 50 to 60 mgm per kilo body weight initially, followed by 0.2 to 0.3 gm every 2 to 4 hours, or at longer intervals. It can be given orally to normal adult males in single dose of 1.0 gm. for 10 days without any untoward reaction. Length of treatment varies in different infections. For example, in scrub typhus, the total dose required is 5 to 6 gm. which is given over the first 12 to 18 hours. In typhoid fever, on the other hand the treatment should be continued for at least 8 days. During the first day, the patient should get from 3 to 6 gm—the total dose required is 18 gm or more. Relapse may follow the use of smaller doses. It is absorbed rapidly from the gastro-intestinal tract of man and excretion or inactivation of the drug occurs rather rapidly. Hence in order to maintain appreciable levels of the antibiotic in the blood, frequent administration of the drug is indicated.

Blood concentration.

Experimental studies show that after an initial dose of 1 gm followed by 0.2 gm every 4 hours, or an initial dose of 2 gm followed by a single dose of 0.5 gm 8 hours later gave maximum blood levels at the end of 2 hours (approximately 6 gamma per ccm). These levels steadily declined to a level not detectable after 8 hours. The drug was excreted by the kidneys and urine levels after 2 hours were approximately 200 gamma per ccm and declined to approximately 50 gamma per ccm, remaining at that level for the next ten days. Such levels produce no significant changes in the blood or the kidneys. No symptoms or signs of toxic effect attributable to the drug were observed.

In white mice, maximum tolerated dose of chloromycetin is 200 mgm per kilo. LD_{50} is 245 mgm per kilo after oral administration. Rabbits tolerate 100 mgm per kilo per day given in 2 daily subcutaneous injections over a period of 8 days. In dogs doses ranging from 72 to 88 mgm per kilo per day in 2 divided doses produced slight gain in body weight; some anaemia developed in animals with this dosage. A dose of 143 mgm per kilo daily produced slight loss of weight, but no changes in temperature and pulse rate, red cell count or hæmoglobin value. The urine of all animals remained free from albumin and sugar. Chloromycetin appears to be fairly rapidly excreted or inactivitated in animals.

(4) *Indications for use*—

(a) Typhus fever—In Typhus fever in which *Rickettsia prowazeki* and *R. mooseri* are the etiological organisms, it is recommended that an initial dose of 60 mgms per kilo body weight be followed by 0.25 gm every 3 hours. With this dosage the temperature and pulse rate were generally found to return to normal within 24 to 48 hours and the symptoms were relieved within 36 to 72

hours. Smadel, Woodward et al (1948) in a report on 25 cases of scrub typhus caused by *R. tsutsugamushi* in Malaya showed that chloromycetin therapy resulted in rapid and complete recovery in all the patients so treated. The dosage recommended is 60 mgm per kilo initially followed by 0.25 gm every 3 hours for seven doses or until the temperature falls to normal.

(b) Scrub Typhus—In the army in Kashmir, cases of scrub typhus rapidly yielded to a total dosage of 3 gm of chloromycetin. An initial dosage of 60 mgm per kg of body weight is recommended followed by 0.25 gm every 3 hours for 7 doses or until the temperature has become normal.

(c) Rocky Mountain Spotted fever—Dramatic evidence on the beneficial effect of chloromycetin therapy was obtained in a series of cases of Rocky Mountain Spotted fever. The temperature fell to normal within 76 hours and other symptoms such as headache and mental confusion started improving on the second day of therapy. The initial dose of 60 mgm per kilo body weight should be followed by 0.25 gm every 3 hours till the temperature has been normal for 48 hours.

(d) Typhoid fever—In typhoid fever chloromycetin has yielded the most satisfactory results. The temperature begins to fall within 24 to 48 hours and returns to normal in 3 to 4 days. Symptoms begin to improve even more rapidly and patients generally feel comfortable after 24 hours, resolution is obvious after 48 hours. An initial dose of 50 mgm per kilo body weight is followed by 0.25 gm every 2 hours until the temperature has returned to normal, after which 0.25 gm may be given every 3 or 4 hours for another five days. Blood culture in some cases become negative within 4 days of treatment. In some of the cases reported on clinical trials, the average total dose of chloromycetin used is between 18 to 24 gm. Toxic symptoms are noted in nearly half of the cases and included nausea, vomitting, distension, diarrhœa, retention of urine, delirium, convulsions, rash and stomatitis.

(e) Undulant fever—Patients with active undulant fever have been favourably influenced by chloromycetin therapy, although the response is not so good in the chronic diseases. The usual initial dose of 60 mgm per kilo body weight, is followed by 0.25 gm every 3 hours for at least 7 days of normal temperature.

(f) Primary atypical (virus) Pneumonia—Chloromycetin has been used with success in a limited number of cases of primary atypical (virus) pneumonia. The dosage employed has been 0.5 gm every 2 to 3 hours for the first 6 doses, and then 0.5 gm 4 times daily. The temperature may fall to normal with in 36 to 48 hours and it is advisable to continue the drug for another 3 to 5 days to prevent relapse.

(g) Bacillary urinary infections—*In vitro,* the drug has favourable effect on the colon group of organisms. *In vivo,* with an oral dosage of 2 to 3 gm. daily, divided into 2 to 4 parts daily, the urine has been found to be free from bacteria in 1 to 3 days. The drug should be continued in dosage of 0.25 gm. 3 to 4 times daily for 5 to 7 days after the urine has become free from bacteria.

In bacillary infections of the urinary tract, infections with *E. coli, Aerobacter aerogenes, Pseudomonas aeruginosa, Klebsiella Pneumonia, Salmonella schottmuleri* and *B. proteus* have been found to respond favourably to chloromycetin. The same initial dosage as above is employed after which 0.25 gm 3 or 4 times a day should be continued for 5 to 7 days after the urine is bacteria free.

5. Polymyxin

It is an antibiotic substance in the metabolic products of *Bacillus polymyxa*, an organism which is relatively common in soil. The name is given as 'Aerosporin' by the Wellcome Physiological Research Laboratories and 'Polymyxin' by the research workers of the American Cyanamid Company. Wellcome research workers later named their original product as Polymyxin A, instead of 'Aerosporin', while the American Cyanamid product is distinguished as Polymyxin D. All the substances are similar in showing high activity against gram-negative organisms, including *Haemophilus pertusis* and *Klebsiella pneumonia.*

Activity.

In vivo experiments on mice with *Eberthela typhosus* or *K. pneumonia* showed that Polymyxin D has about 1/10th the activity of the A form, but the toxicity of A form was found to be about 3 times that of D form. The main difference in chemistry between the two forms is that D contains the amino acid series. All antibiotics of the series appear to be basic polypeptides, some or all of which contain L-a:y-diaminobutyric acid, pleucine, L-threonine, and a fatty acid of the empirical formula C_9 H_{18} O_2. At John Hopkins Medical School, Polymyxin D was found to be more toxic than Streptomycin in experimental animals on a weight basis; on the other hand, it was also considerably more effective against *K. pneumonia.* Against gram-positive pneumococcus type 1 in mice it was relatively ineffective. The toxic action was found to be exerted mainly on the tubular epithelium of the kidneys. *In vitro* the substance is active against a wide range of gram-negative organisms. No induced resistance to the antibiotic was observed. The Wellcome group reports that the A compound is superior to the D forms in experimental whooping cough infections and in acute infections with *Eberthela typhosus.* At John Hopkins Medical School the doses used was either 3 mgm/kg body weight intramuscularly per day in 8 doses or 7 mgm/kg per day in 3 doses. The latter was not tolerated for more than 2 to 3 days. Treatment was usually continued for 5 to 10 days. Of the 22 patients treated, the clinical results are described as good or excellent in those with infections due to *Pseudomonas aeruginosa, H. pertusis, Aerobacter aerogenes, K. pneumonia, E. coli* and in Brucellosis. In pertusis the results are particularly striking with small infants who are seriously ill. Good results are also quoted in infantile gastro-enteritis when the drug is given orally. In severe instances of such infections the total daily dose of polymyxin has been based upon 3 to 5 mgms of the antibiotic per kg of body weight, this being split into 8 equal doses and given in a special buffer solution (pH 7.4) at interval of 3 hours.

Doses.

Toxicity.

Polymyxin is moderately toxic for white mice, the LD_{50} being 250 to 300 milligram per kilogram when given by a single subcutaneous injection. In larger animals such as dogs 10 milligram of polymyxin D per kilogram given twice a day for 7 days by the intramuscular route was well tolerated. All samples of polymyxin tested have produced albumin, casts, white and red blood cells in the urine of rats which had received 20 mgm per kgm of polymyxin daily by the intravenous route.

Absorption.

Polymyxin passes readily into the blood stream following its intramuscular injection into dogs. When polymyxin was administered to human beings in devided doses by the intramuscular route at intervals of 3 hours in amounts not to exceed a total daily dose of 3 mgm per kgm of body weight, concentrations of 0.6 to 1.3 micrograms of the antibiotic per c.c. were noted in the serum after 24 hours of therapy. Detectable amounts of the antibiotic were present in the urine of these patients.

Toxic manifestation.

All specimens of this antibiotic which has been tested have produced varying degrees of renal tubular dysfunction. In the mildest form it appears as a fixation of the specific gravity of the urine, while in severe forms, oliguria with albumin, casts, red blood cells and white cells in the urine, an elevated non-protein nitrogen, and depressed renal function may occur. These latter reactions sometime become very disturbing and it is because of them that the general use of polymyxin seems contra-indicated. Histamine like reactions, hyperesthesias and fever have been noted in the course of polymyxin therapy, but these reactions would appear to be due to impurities in the specimens of polymyxin which were being used, rather than to the antibiotic itseif.

6. Tyrothricin (Gramicidin & Tyrocidine)

When bacillus brevis is grown in one per cent tryptone broth for four days at 37°C it produces a substance which was first called gramicidin. This substance along with tyrocidine was later known as tyrothricin, which has bactericidal properties against most gram-positive coccal pathogens with the possible exceptions of strains of *Staph. aureus.* It also, under certain conditions, acts on certain gram-negative species. Tyrothricin is effective in the treatment of experimental infections with hæmolytic streptococcus. Both gramicidine and tyrocidine hydrochloride are crystalline substances which are stable in alcohol even at high temperature; they are feebly soluble in water, but fairly stable in colloidal emulsions. *Str. fæcalis* and related fæcal streptococci are sensitive to the action of tyrothricin; it, therefore, exercises a selective action. In animal experiments tyrothricin and its components are only effective when placed in direct contact with the infecting bacteria and not when they are given by the mouth or systemically when the infection is given intraperitoneally.

Haemolysis.

Toxicity.—It has been shown that both fractions of tyrothricin produce hæmolysis when given by the intravenous route and this action precludes their successful use against organisms circulating in the blood. Efforts to get rid of hæmolytic effect have not been successful so far. Daily intravenous injections of 2.0 mgm of gramicidin or tyrothricin per kiio body weight produced death within 2 to 8 days, the animals losing appetite for food, loss in weight and the red blood cells fell from 6 million to 2½ million per ccm. All these substances are definitely toxic on intraperitoneal and intravenous injections but not when given by mouth. They are therefore, destroyed in the gastro-intestinal tract or are not absorbed.

Cytoplasmic and nuclear changes.

Tyrothricin and tyrocidine in high concentrations produce cytoplasmic and nuclear disintegration of polymorphonucleur leucocytes, though no apparent injury occurred to the cells and they continued to phagocytose bacteria. Gramicidin is more toxic in this respect than tyrocidine and the cytotoxicity of tyrothricin is largely due to its gramicidine content. Acute toxicity of tyrothricin and its fractions has been observed in rabbits and dogs, death resulting within a few days when it is given intravenously. Tyrothricin and its two fractions have no marked antibacterial properties and when used locally (in alcoholic or aqueous solution) do not produce serious toxic effects.

Of the three substances, tyrothricin is the most suitable for local use as it is fairly easily prepared and easily obtainable. There is some selective difference between the action of gramicidin and tyrocidine, the latter being slightly active against gram-negative organisms.

Use in infected ulcers.

The most satisfactory responses were in cases in which ulcers are infected with *Str. pyogenes;* in the treatment of infections with *Staph. aureus* the results were less satisfactory. Tyrothricin is also of definite value in the treatment of certain types of infected ulcers of ischæmic and stasis origin. For small ulcers, a sterile cotton pad is saturated with the aqueous solution with a dropper every hour, the pad was changed every 24 hours or after if there was much discharge. Tyrothricin is useful in preparation for skin grafting of infected ulcers and wounds with *Str. pyogenes* and *Staph. aureus;* the infecting organisms are thus eliminated.

7. Gramicidin S

Gramicidin S is produced from *B. brevis var. gause-brazhinkova.* Improvements have been made during recent years on large-scale production of gramicidin S (from rough variants of *B. brevis* (on yeast autolysate with tryptone) in U.S.S.R. and the yield is now 3.0 gm of crystaline material per litre of nutritive medium. A study of its chemistry has shown that it has a simple composition (consisting of five different amino-acid residues).

Gramicidin S has a more potent action against Staphylococci than tyrothricin, gramicidin and tyrocidine hydrochloride. It has a selective action on gram-negative bacteria. Dysentery group of bacilli are very susceptible to its lethal action whereas typhoid and para-typhoid bacilli (with the exception of *Bact. para-typhoid* A) are resistant to it. Its action on these organisms is reduced in the presence of blood. It kills enterococci which play an important part in septic processes, and which are resistant to penicillin and sulphonamides. Experiments have shown that epithelisation of wounds treated with gramicidin S is more rapid. Its application to wounds prevents suppuration effectively. It is now being used in surface wound dressing.

8. Streptothricin

Preparation.

The substance was first discovered in 1942, and in addition to its antibacterial activity against certain gram-positive pathogenic bacteria it is also active against certain gram-negative organisms. Streptothricin has been isolated from soil organism of the genus Actinomyces identified as *Actinomyces lavendulae.* It is produced when the organism is grown in a tap water medium containing 1 per cent glucose or starch, 0.5 per cent tryptone, 0.2 per cent potassium phosphate, 0.2 per cent sodium chloride, 0.001 per cent ferrous sulphate and 0.25 per cent agar. This substance is readily soluble in water, dilute mineral acids but is destroyed by concentrated acids. It is insoluble in ether, petroleum ether or chloroform. From the tryptone medium it is precipitated by substances which precipitate proteins but it has not the characteristics of proteins. On electro-dialysis the active substances is found to move to the cathode at a pH of 7 and behaves as an organic base. Streptothricin is produced much more readily and abundantly under conditions of submersion combined with agitation and aeration.

Activity against gram-negative pathogens.

Activity of Streptothricin in Vitro and in Vivo—Streptothricin possesses bactericidal properties especially against certain gram-negative bacteria, *e.g., E. coli.* It offers some promise as an bactericidal agent against brucellosis in animals, 0.1 mgm of crude streptothricin per 10 ccm of broth preventing growth of this organism. In guinea pigs infected with *Brucella abortus* 600 mgm of crude streptothricin sterilized the blood. Streptothricin inhibited completely the activity of the group of Eberthella typhii. The genus clostridium (tetani, welchii, septique and sordeli) and certain other organisms such as *B. pyocyanous, B. proteus, Str. viridans* and *Str. lactis* were also resistant to its action.

Local and general use.

Streptothricin is suitable for application to infected wounds and mucous membranes and 0.5 to 1.0 per cent solution in normal saline does not injure the conjunctiva. This substance should prove useful in the local treatment of infected wounds and burns. Unlike other antibiotic agents, streptothricin can be given orally and markedly decreases the number of lactose-fermenting bacteria in the intestinal canal. It is, therefore, believed that it may be of value in the treatment of certain types of dysentery and other infections caused by susceptible bacteria (para-typhoid organisms, etc). It is, however, not adivisable to give it by the intravenous or subcutaneous routes in the treatment of systemic infections.

Estimation of potency.

As in the case of penicillin, the unit of streptothricin is the amount by weight of streptothricin which will completely inhibit the growth of the test organism (bacillus subtilis in this case). The unit of streptothricin is the minimum quantity of the drug which when added to one ccm of nutrient broth will inhibit growth of inoculum of *E. coli.*

Possible clinical use.

Streptothricin given subcutaneously or intravenously to experimental animals may at times produce either early or delayed death. It is not, therefore, suitable for systemic use.

9. Circulin

As recently announced by Murray and Tetrault (1948), it is an antibiotic which is more active against gram-negative than gram-positive bacteria and has been obtained from a strain of bacteria closely resmbling *Bacillus circulaus.* It is very soluble in water, soluble in alcohol but insoluble in the hydrocarbons. The antibiotic retains its activity when stored at a pH of 2.5 to 6.5 at 4°C for at least 3 months and is also stable to autoclaving for 15 minutes at 15 pounds pressure in the same pH range. This antibiotic has a bacterial activity similar to polymyxin and aerosporin but it is less active than polymyxin against gram-negative bacteria, but more active than polymyxin against gram-positive bacteria. Circulin is inactivated by crude trypsin whereas polymyxin is not. Circulin appeares to be somewhat less toxic than aerosporin but more toxic than polymyxin. It is active in vivo against *S. typhosa* and *Klebsiella Pneumonia.*

10. Subtilin

Subtilin, an antibiotic agent discovered by Jansen and Hirschmann (1944). Salle and Jann (1945) reported that subtilin in dilution of 1 : 50,000 prevented surface growth of *Mycobacterium tuberculosis* on Long's synthetic medium. They concluded that 'subtilin is bacteriostatic in high dilution and germicidal in greater concentrations'.

Subtilin had a moderate inhibitory effect on the growth of the H37 Rv strain of the tubercle bacillus in sorbitan monooleate albumin liquid medium, but very little effect in the other three media that did not contain sorbitan monooleate. Subtilin did not influence the course of a tuberculous infection in guinea pig produced by the H37 Rv strain of tubercle bacillus.

11. Bacitracin

Bacitracin, an antibiotic produced by strains of *bacillus subtilis*, was used in 105 cases of surgical infection, refractory to sulpha drugs or other antibiotics. There was favourable response in 70 per cent of the cases and in 1/5th of these the results were spectacular. Three cases of extensive progressive gangrene responded within 72 hours and recoverd with surgical excision. Most of the cases of cellulitis, deep abscess and infected accidental wound made a favourable response. A considerable number of staphylococcal strains are noticed resistant to penicillin but susceptable to bacitracin. The cases that exhibited spectacular response was mostly of staphyloccal and streptococcal (haemolytic, non-haemolytic, micro-aerophilic, and anaerobic streptococci). Some of the later preparations of bacitracin made by the deep tank method were toxic to kidney; so it has been advised that renal function tests should be performed when bacitracin is used systemetically.

12. Biocerin

Strains of *Bacillus cereus* isolated from milk was reported to exhibit marked antagonism to both gram-positive and gram-negative organisms, e. g. salmonella and shigella. The antibiotics substances reported in this study were produced by an organism isolated from the soil and identified by following a key to the identification of ærobic sporeforming organisms, as *bacillus cereus.*

Biocerin was produced by growing *Bacillus cereus* in a medium containing glucose 20 g, $NH_4H_2PO_4$ 1.0 g. Methionine 0.005 g, Agar 5.0 g, distilled water 1,000 ml. final pH=7.0. All bacterial species tested are inhibited by a concentration of 1.0 mg. of crude biocerin per ml. of agar.

Salmonella paratyphosus A, Salmonella paratyphosus B, Staphylococcus aureus and *Staphyllococcus albus* were not inhibited by a concentration of a 0.5 mgm of crude biocerin per ml. of agar.

The toxicity of crude biocerin was determined by suspending the material in sterile mineral oil and inject intraperitoneally into white mice weighing approximately 18-20 gms. A single injection of 20 mgm of crude substance produced no fatalities in a group of 10 mice at the end of a 5 day test period.

13. Miscelianeous Antibiotics

Pyocyanase and pyocyanine.

Pyocyanase is a thermostable substance of lipoid nature. Pyocyanine is a blue pigment soluble in chloroform. Although these substances were active against certain gram-positive and gram-negative pathogens, they were found to be very toxic in experimental animals; intra-peritoneal injection of 2 mgm of pyocyanine produced death in the animal.

Actinomycin A and B.

Actinomycin A is produced by Actinomyces antibioticus and is an orange coloured highly toxic substance soluble in ether or alcohol. Actinomycin B is soluble in ether and insoluble in alcohol. Both are exceedingly toxic.

Actinomycin A is producced by *Actinomyces antibioticus* and is an orange coloured substance readily soluble in water. It is active against gram-positive bacteria and colon-typhoid-salmonella group of gram-negative organism. Little is known about its therapeutic value.

Citrinin.

Citrinin is produced by *penicillium citrium* and is a yellow substance which is toxic to animals when given by the mouth or intraperitoneally.

Gliotoxin is produced by *Trichoderma lignorum*. It is active against certain gram-positive organisms and also against gram-negative pathogens. It is toxic and holds little promise as a chemotherapeutic agent. *Gliotoxin.*

Fumigatin is derived from *Aspergillus fumigatus*, is soluble, and is very active against certain gram-positive organisms. *Fumigatin.*

Claviformin is obtained from cultures of *Penicillium claviforme*, clavacin from *Aspergillus clavatus* and patulin from *Penicillum patulum*. They all have the same empirical formulla. Patulin has been tried with beneficial results in the treatment of common colds, 1 in 10,000 solutions being sprayed into the nose or snuffed up from the hand. None of these substances are suitable for subcutaneous or intravenous administration on account of their high degree of toxicity. *Claviformin, Clavacin and Patulin.*

Fumigacin and helvolic acid are produced by *Aspergillus fumigatus*. In pure form fumigacin occurs as fine white needles. Both these substances are toxic to experimental animals. *Fumigacin and helvolic acid.*

Aspergillic acid was obtained from *Aspergillus flavus* and occurs in crystaline form. It is too toxic for systemic use but it may be useful for local application in gas gangrene. *Aspergillic acid.*

Flavicin was also isolated from *Aspergillus flavus*. It is soluble in water and ether and has low toxicity and high antibacterial activity in the same way as penicillin. There is, therefore, possibility of another therapeutic agent comparable with penicillin. *Flavicin.*

Gignatic acid is obtained from *Aspergillus gignateus*. It closely resembles penicillin in its properties. *Gignatic acid.*

Flavicidin and penicillin protect mice to an equal degree against pneumococcal infection. It is highly soluble and readily absorbed by the body tissues; it is excreted by the kidneys. The organisms rendered resistant to penicillin were also found to be resistant to flavicidin. *Flavicidin.*

Antibiotic substances of non-microbial origin.

The antibiotic substances described above are derived from micro-organisms. The following antibiotic agents are derived from other natural sources:—

Lysozyme, chlorellin, canavalin and allicin.

Lysozyme was first discovered by Fleming in 1922 in the nasal secretions of a patient suffering from coryza. He also showed that nasal mucus possesses a powerful inhibitory and lytic action on different organisms and that this action was also possessed by some animal and vegetable tissues and various secretions of the human body. Lysozyme occurs in tears, sputum, saliva, blood serum and plasma, peritoneal fluid, effusion, sebum, semen, etc., but is not found in normal urine, cerebrospinal fluid or sweat. It is stable and maintains its activity in fluids for many weeks the dry material also does not deteriorate. Lysozyme occurs in large quantities in commercial dried egg albumin prepared weeks and months ago. It is soluble in water, insoluble in alcohol, ether, chloroform, acetone, xylol, etc. The substance appears to be of a protein nature of low molecular weight. It has the power of splitting carbohydrates and belongs to the class of carbohydrates. It is not of any marked significance as a chemotherapeutic agent. *Lysozyme.*

Chlorellin was obtained from unicellular algae, *Chlorella vulgaris* and *Chlorelia pyrenoidosa* when cultured in solution of mineral nutrients used for algal cultures. This substance inhibits growth of staphylococci and of *Str. pyogenes* as well as growth of gram-negative *E. coli*. The presence of rabbit's serum does not interfere with its antibacterial activity. Further studies are being carried out with regard to its therapeutic and toxic properties. *Chlorellin.*

Canavalin is obtained from soyabean or jack bean flour in a mixture of water and organic solvents such as alcohol. It appears that canavalin is effective against *Staph. aureus* and inhibits the growth of gram-positive and gram-negative organisms. It has been tried in the treatment of penumococcal pneumonia with good results, the average dose per day being 30 ccm given by intravenous drip method. Further work is in progress.

Allicin is the antibacterial principle occuring in *Allium sativum* (the common garlic). The compound is said to contain about 40 per cent of sulphur, no nitrogen and no halogens, and its action is more of bacteriostatic than bactericidal nature. The substance is equally *Allicin.*

effective against gram-positive and gram-negative organisms. Its effectiveness against *Staph. aureus* is equal to approximately 15 units of penicillin per mgm and its activity is not affected by the presence of para aminobenzoic acid. In dilutions of 1 in 125,000 this substance inhibits the growth of large number of gram-positive and gram-negative organisms including *Staph. aureus, Str. viridans, Bacillus subtilis, Eberthella typhi* and other members of colon-typhoid-dysentery group. It remains to be seen whether it will be of any chemotherapeutic value or not.

Other antibiotics.

A number of other antibacterial agents or antibiotic substances occur in nature and certain flowering plants and weeds are found to contain them. There is a very large field waiting for the investigation of these.

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ERRATA

Page	Para	Line		Corrections
3	4	4	.....	For '6×10' read '6×10^{23}'
21	5	5	.....	For 'atoniseris' read 'atomiser is'
48	4	2	.....	For 'grain' read 'gram'
49	2	4	.....	After 'increase' read 'this'
66	last	2	.....	For 'distention' read 'tension'
74	5	13	.....	After 'pressure' read 'are present'
91	2	7	.....	After 'taken' read 'for its'
91	4	2	.....	For 'sandards' read 'standards'
129	1	3	.....	For 'hydroscopic' read 'hydropic'
152	1	2	.....	For 'lessions' read 'lesions'
206	3	2	.....	After 'female' read 'is'.
256	1	1	.....	For 'non' read 'uni'
256	under classification of protozoal			The names of species are misplaced—not opposite the proper genus, read opposite respectively.
256	under classification of protozoal—No. II.			For 'mans' read 'means'
270	side heading	.....	.....	With 'differential' read 'diagnosis'.
276	7	7	.....	For 'powders' read 'pills'
293	last	6	.....	After 'tablets' read '.' (Full stop).
303	7	3	.....	For 'ovala' read 'ovata'
304	side heading	.....	.....	For 'clinical' read 'chemical'
316	3	16	.....	For 'gm' read 'mgm'
332	4	3	.....	For 'Sb iv' read 'Sb iii'
337	last para	last line	.....	for '0.25' read '0.025'
341	3	16	.....	For 'different' read 'difficult'
346	last para	last line	.....	After 'route' read 'large'
350	1	3	.....	For 'gm' read 'out'
350	1	5	.....	For 'gm' read 'mgm'
352	3	1	.....	After 'potassium' read 'iodide'
353	3	1	.....	For 'amomatic' read 'aromatic'
358	1	4	.....	For 'which' read 'schizogony'
371	2	3	.....	For 'Racial' read 'Radical'
377	under species of cinchona		.....	For 'crown' read 'brown'
418	2	7	.....	Omit 'count' after 'hæmoglobin'
422	5	5	.....	For '6' read '3'
432	1	10	.....	For 'calls' read 'cells
436	6	3	.....	For 'rate' read 'rats'
437	1	7	.....	For 'rage' read 'range'
438	1	1	.....	For 'sanctochin' read 'santochin'
471	2	2	.....	For 'round' read 'found'
483	1	1	.....	For '30,000' read '300,000'
504	last	1	.....	For '0.06' read '0.6'
517	2	3	.....	For '20' read '2.0'
519	2	1	.....	After 'this' read 'is'
535	2	7	.....	For 'lancoevtes' read 'leucocytes'
557	2	2	.....	For 'Diseases' read 'cases'
562	5	last	.....	After '12' read 'KK'
594	4	18	.....	For '300' read '30.0'
614	last	1	.....	For 'Phthaly' read 'Phthalyl'
620	3	7	.....	For "enables the maintenance of adequate concentration in the affected" read "and epileptiform symptoms may be produced. Sulphadiazine is"

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08

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